SYNTHESIS OF 6-ALKYL DERIVATIVES OF DOPA VIA THE TRANS ISOMERS OF UNSATURATED AZLACTONES

A. P. MORGENSTERN
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Aan mijn ouders
Aan mijn vrouw
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CHAPTER I

INTRODUCTION

In 1911 Funk\(^1\) postulated that 3-(3, 4-dihydroxyphenyl)alanine (dopa) would be an intermediate product in the biosynthesis of adrenaline from phenylalanine or tyrosine. This presumption has since been experimentally confirmed; the major pathway\(^2\), proposed for catecholamine formation in the mammal, is indicated in scheme 1.

**SCHEME 1** Biosynthesis of catecholamines

![Chemical structure diagram showing the biosynthesis of catecholamines from tyrosine to adrenaline.](attachment:Chemical_Structure_Diagram.png)
The enzymes involved in the scheme are: (a) tyrosine hydroxylase, (b) dopa decarboxylase, (c) dopamine-β-hydroxylase, (d) phenylethanolamine-N-methyl transferase.

Especially its role as a precursor of catecholamines has made dopa the subject of numerous experiments. In 1966 Patel and Burger\textsuperscript{3}) gave a survey of this work (752 lit. ref.).

The conversion of dopa into dopamine is catalyzed by dopa decarboxylase; an enzyme that is also responsible for the decarboxylation of ortho- and meta-tyrosine and 5-hydroxytryptophan\textsuperscript{4}). Only the L-isomers of the amino acids are involved in the enzymatic reactions. The inhibition of dopa decarboxylase has been the subject of extensive studies, based on the assumption that such investigations may lead to successful treatment of clinical conditions where a decreased production of noradrenaline would be beneficial\textsuperscript{2}). Compounds, structurally related to dopa, occupy an important position in the large group of dopa decarboxylase inhibitors. Dopa derivatives carrying alkyl substituents in the amino acid chain and in the benzene nucleus have been reported in the literature.

\[
\begin{align*}
\text{HO} & \quad \text{COOH} \\
\text{HO} & \quad \text{CH}_{2} \\
\text{CH} & \quad \text{CH} \\
\text{NH}_{2} & \\
\end{align*}
\]

The most important alkyl-dopa is α-methyl-dopa, first synthesized around 1945\textsuperscript{5}) and since then studied by many authors\textsuperscript{6}). Experiments in vitro and in vivo have revealed that α-methyl-dopa is an active inhibitor of dopa decarboxylase; the amino acid itself is also decarboxylated by the enzyme, thus giving rise to α-methylated metabolites. It has been suggested recently\textsuperscript{2,7}) that the action of these metabolites on the tissue stores of noradrenaline ("false transmitter hypothesis") is responsible for the antihypertensive effect of α-methyl-dopa, a property that makes this compound of great therapeutic importance.

In vitro, α-ethyl-dopa is a somewhat weaker inhibitor than α-methyl-dopa, the amino acid not being decarboxylated by the enzyme\textsuperscript{8}).

The α-substituted propyl and butyl compounds are claimed to be antihypertensive agents\textsuperscript{9}); there are no literature data showing their activity as a dopa decarboxylase inhibitor. The same is true for the α,β-dimethyl\textsuperscript{10}) and the α-ethyl-β-methyl derivative\textsuperscript{11}) and for analogues of α-methyl-dopa, obtained by incorporation of the α- and β-carbon atoms in an aliphatic ring system\textsuperscript{12,13}).
Alkyl substitution in the 2- and the 6-position in dopa affords compounds which are interesting with regard to the formation of melanin\textsuperscript{14).} Of the 2-methyl\textsuperscript{15),}\ 2-ethyl\textsuperscript{16),}\ 2-phenyl\textsuperscript{17),}\ 2-benzyl\textsuperscript{17),}\ 6-methyl\textsuperscript{18)}\ and 6-propyldopa\textsuperscript{19)} only the methyl-substituted compounds are mentioned in the report\textsuperscript{20)} on experiments with dopa decarboxylase.

In vitro both the 2-methyl and the 6-methyl derivative proved to be substrates; an inhibitory effect could not be demonstrated. It was recently established that the combined presence of an \( \alpha \)-methyl group in the side chain and an ortho-methyl substituent in the benzene nucleus affects the inhibition of dopa decarboxylase in vitro.

Introduction of a 2-methyl or 6-methyl group in \( \alpha \)-methyldopa was found to decrease the inhibitory activity sharply\textsuperscript{21).}

The above literature data do not indicate to what extent increase in bulk of the alkyl substituent in ortho-alkyl-substituted dopa derivatives affects their activity as inhibitors of dopa decarboxylase.

This problem was the subject of the present investigation; chapter 2 and 3 will deal with the synthesis and chapter 5 with the measurements of the activities of 6-methyl-, 6-ethyl-, 6-isopropyl- and 6-tert. butyldopa.

The four amino acids were prepared from the azlactones of the corresponding 2-alkyl-\( \alpha \)-benzamido-4, 5-dimethoxycinnamic acids.

There is a conflict in literature\textsuperscript{22) regarding the geometric configuration of the stable azlactone carrying no substituents in the benzylidene group (4-benzylidene-2-phenyl-2-oxazolin-5-one).

Our NMR spectral studies\textsuperscript{23) on the geometric isomers of \( \alpha \)-benzamido-3, 4-dimethoxycinnamate and their related azlactones (chapter 4) have led to a cis-trans assignment, which is in contrast to recent literature data\textsuperscript{24) .}
LITERATURE

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CHAPTER 2

SYNTHESIS OF 6-METHYL-, 6-ETHYL- AND 6-ISOPROPYLDOPA.

2.1. INTRODUCTION

The methods most widely used in the synthesis of substituted phenylalanine derivatives can be divided into two groups:

A. Aldehyde condensations: the synthesis of the amino acid proceeds via condensation of a glycine derivative with a substituted benzaldehyde. This group includes the Erlenmeyer-Plöchl synthesis of azlactones; its application in the preparation of the title compounds, is discussed in chapter 2.2.

B. Coupling reactions of substituted benzyl halides with aminomalonate derivatives. In this thesis (chapter 2.3) an example is given. The reaction concerned did not, however, yield the desired result.

The selection of the route of synthesis is, as a rule, determined by the accessibility of the benzaldehydes and benzyl halides.

Regardless of the route chosen, it is recommendable to protect ortho-dihydroxy groups in the starting material during synthesis. In the syntheses discussed here, ortho-dimethoxybenzene (veratrole) derivatives were employed because the methyl ethers offer maximal protection with respect to side reactions.

Although the removal of the protecting groups occurs under rigorous conditions, no difficulties were encountered in the synthesis of 6-methyl-, 6-ethyl- and 6-isopropyl-dopa.

The synthesis of 6-tert-butyl-dopa will be dealt with in a separate chapter (3) because of its different course.
4-Methyl- and 4-ethylveratrole were prepared according to literature data\(^1\), by reduction of veratraldehyde and 3,4-dimethoxyacetophenone, respectively. Reaction of methylmagnesium iodide with this acetophenone and subsequent distillation at reduced pressure afforded 3,4-dimethoxy-\(\alpha\)-methylstyrene, which was converted into 4-isopropylveratrole by catalytic reduction. Methylation of the commercially available 4-isopropylcatechol with dimethylsulphate according to Baker\(^3\), appeared to be a more efficient route of synthesis.

\(^{(*)}\)The alanine derivatives, prepared according to this scheme, are equimolecular mixtures of the enantiomorphs. In the synthetic part of this thesis the prefix DL will be omitted.
The great reactivity of phenol ethers towards electrophilic agents allows direct introduction of an aldehydic group in para position. A large number of 6-alkylveratraldehydes (alkyl = unbranched chain) were synthesized by Kachru and Patak\(^4,5\) during their investigations into new amoebacides. The authors introduced the aldehydic group via a Gatterman synthesis with zinc cyanide and hydrogen chloride but obtained yields practically always smaller than 40%. Our attempt to synthesize 6-methylveratraldehyde in this manner afforded only 15% of aldehyde. The Vilsmeier formylation with dimethylformamide and phosphorus oxychloride has already been successfully applied to 4-methylveratrole\(^1\). The 5-position is strongly favoured in an attack by the active electrophilic part of the Vilsmeier reagent (presumably\(^6\)) complexes such as \([\text{Cl}_2\text{PO-O-CH=NR}_2]^+\text{Cl}^-\).

Examples of introduction ortho with respect to the methoxy groups, have not been reported. Byck and Dawson\(^7\) did not succeed in formylating 4, 5-dimethylveratrole.

The present synthesis of the 6-methyl-, 6-ethyl- and 6-isopropylveratraldehydes was brought about by means of the Vilsmeier reaction. Formylation of the isopropylveratrole gave a poor yield (about 20%). However, much of the starting material could be recovered so that the reaction could be repeated. The route via the Grignard compound of 4-bromo-5-isopropylveratrole, as described in this thesis for the synthesis of 6-tert-butylveratraldehyde, afforded a somewhat higher yield (about 30%). The NMR spectra of the three veratraldehydes (see experimental part) showed two singlets for the two aromatic protons. It follows that the two protons must occupy para positions towards each other. In chapter 3 we shall deal with the reliability of a structural assignment based on the absence of a splitting of the signals from two aromatic protons in the NMR spectrum.

Both the synthesis of the azlactones and the hydrolysis of the lactone ring by means of alcoholic potassium hydroxide were carried out according to literature data. In chapter 4 the geometric configuration of the cinnamates and their related azlactones are discussed in detail.

It is possible to convert the \(\alpha\)-benzamidocinnamic acid into the desired amino acid in one step. Cleo\(^8\) and Cromartie\(^9\) made use of hydroiodic acid with red phosphorus in acetic anhydride in the synthesis of dopa derivatives; both the reduction of the double bond in the cinnamic acid and the removal of the pro-
tecting groups could be effected with the aid of this reagent. However, when using hydroiodic acid and red phosphorus in the synthesis of alkyl-substituted tyrosines, Nikiforov\textsuperscript{10}) could not isolate any amino acid, which is in agree-
ment with our experiences in the preparation of 6-methyldopa.

Hydrogenation of the double bond, followed by isolation of the substituted dihy-
drocinnamic acid and removal of the protecting groups, was found to be the
most reliable method in the syntheses described here.

Catalytic hydrogenation appeared to be an efficient method to convert the α-
benzamidocinnamic acid into the dihydro analogue. Both hydrogenation with
Raney nickel at a hydrogen pressure of ca. 30 atmosphere and the addition of
the nickel-aluminum alloy according to Nikiforov\textsuperscript{10}) gave good results. In the
case of the ortho-isopropyl-substituted derivative the latter method was pre-
ferred because it gave better yields. From the U.V. spectrum\textsuperscript{11}) of the reac-
tion product it could be clearly established whether there was any cinnamic
acid left.

The protecting groups (O-methyl and N-benzoyl) in the hydrogenated product
were removed by heating with concentrated hydrobromic acid as described by
Cromartie\textsuperscript{12}) and Burger\textsuperscript{13}).

\section*{EXPERIMENTAL PART}

All melting points - obtained with a Reichert microscope with Kofler heating- are uncorrected. The
IR spectra were measured with a Perkin-Elmer (237) spectrofotometer and the UV-visible spectra
with a Perkin-Elmer (137 uv) apparatus. The NMR spectra were determined on a Varian A-60A at ca.
38\textdegree. The chemical shifts are expressed in δ values (p.p.m.) relative to a tetramethylsilane (TMS)
internal standard; when the spectrum was measured in D$_2$O, sodium 3-(trimethylsilyl)-propanesulfonate
was used as a standard.

4-Methylveratrole. Synthesized according to Bruce\textsuperscript{11}):
b.p. 104-106\textdegree/13 mm; n$^20$: 1.5290; yield 85%.

4-Ethylveratrole. Synthesized according to Barger\textsuperscript{2}):
b.p. 115-117\textdegree/15mm; n$^20$: 1.5228; yield 65% from 3,4-dimethoxyacetophenone\textsuperscript{14, 15}.

4-Isopropylveratrole. The catechol was methylated according to Baker\textsuperscript{30}) under N$_2$ in an-
hydrous acetone-benzene-K$_2$CO$_3$ with vigorous stirring. In order to remove the small amount of
alkyl guaiacol, as a rule still left in the crude distilled alkylveratrole, the crude oil was
dissolved in some benzene or toluene and vigorously stirred with 10-20% aqueous KOH at
room temperature for 2 hr. After separation of the organic layer, washing and distillation,
a product was obtained that no longer showed an OH-band in the IR spectrum at ca. 3500 cm$^{-1}$.
b.p. 117-120\textdegree/11 mm; n$^20$: 1.5170; yield 80%.
6-Methylveratraldehyde. Synthesized according to Bruce\textsuperscript{1}; b.p. 115-120\textdegree/1 mm; m.p. 72-74\textdegree (lit. 72-73\textdegree); yield 62%; NMR (CDCl\textsubscript{3}): 6 2.58(CH\textsubscript{3}), 3.86 and 3.89 (6 H, OMe), 6.65 (singlet, IH, aryl CH), 7.28 (singlet, IH, aryl CH), 10.12 (IH, CHO).

The following compounds were formed by the same route:

6-ethylveratraldehyde; b.p. 125-130\textdegree/1 mm; m.p. 24-26\textdegree (lit. 16) ca. 26\textdegree; yield 45%; m.p. semicarbazone 197-199\textdegree (lit. 16) 198\textdegree; NMR (CDCl\textsubscript{3}): 6 1.28 and 3.01 (CH\textsubscript{2}CH\textsubscript{3}), 3.89 and 3.93 (6 H, OMe), 6.70 (singlet, IH, aryl CH), 7.34 (singlet, IH, aryl CH), 10.21 (1 H, CHO).

6-isopropylveratraldehyde; the crude aldehyde, obtained by extraction and subsequent distillation, was purified via the bisulphite addition compound. B.p. 121-128\textdegree/10\textsuperscript{-2} mm; \( \nu \text{cm}^{-1} \): 1.5610; yield 20%; NMR (CDCl\textsubscript{3}): 6 1.27 and ca 3.9 ((CH\textsubscript{3})\textsubscript{2}CH), 3.83 and 3.88 (6 H, OMe), 6.80 (singlet, IH, aryl CH), 7.24 (singlet, IH, aryl CH), 10.18 (1 H, CHO).

Analysis semicarbazone (m.p. 178-181\textdegree):

calc. for C\textsubscript{13}H\textsubscript{19}N\textsubscript{5}O\textsubscript{3}: 58.86\% C, 7.22\% H, 15.84\% N
found: 58.7 \% C, 7.3 \% H, 16.0 \% N.

4-(4, 5-Dimethoxy-2-methylbenzylidene)-2-phenyl-2-oxazolin-5-one.

Synthesized as described\textsuperscript{17} for the unsubstituted dimethoxy-benzylidene-oxazolone. The deep yellow crystals had a m.p. 168-169.5\textdegree (lit. 12) 167-168.5\textdegree. Yield 72%; NMR see chapter 4.

Anal. calc. for C\textsubscript{19}H\textsubscript{17}NO\textsubscript{4}: 70.57\% C, 5.30\% H, 4.33\% N
found: 70.4 \% C, 5.3 \% H, 4.3 \% N.

The following compounds were prepared by the same route:

4-(2-ethyl-4, 5-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-one;

deep yellow crystals with a m.p. 155-157\textdegree (lit. 2) 155\textdegree; yield 57%; NMR see chapter 4.

Anal. calc. for C\textsubscript{20}H\textsubscript{15}NO\textsubscript{4}: 71.19\% C, 6.68\% H, 4.15\% N
found: 71.3 \% C, 5.7 \% H, 4.4 \% N.

4-(4, 5-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-one;
appearing as deep yellow crystals; m.p. 162-164\textdegree; yield 53%; NMR see chapter 4.

Anal. calc. for C\textsubscript{21}H\textsubscript{21}NO\textsubscript{4}: 71.77\% C, 6.02\% H, 3.99\% N
found: 71.4 \% C, 6.1 \% H, 3.9 \% N.

\( \alpha \)-Benzamido-4, 5-dimethoxy-2-methylcinnamic acid. Synthesized according to Cromartie\textsuperscript{12}; m.p. 214-216\textdegree (dec.) (lit. 12) 212-214\textdegree; yield 84\%; UV (ethanol): maxima at 298 and 329 mum; NMR (D\textsubscript{6}-acetone): \( \delta \) 6.93, 7.36 and 7.76 (3 singlets for 3H, aryl CH and olefinic H), 3.59 and 3.88 (6H, OMe), details are given in chapter 4.

Anal. calc. for C\textsubscript{19}H\textsubscript{15}NO\textsubscript{5}: 66.85\% C, 5.61\% H, 4.10\% N
found: 66.6 \% C, 5.5 \% H, 4.0 \% N.

The following compounds were obtained in the same manner:

\( \alpha \)-benzamido-2-ethyl-4, 5-dimethoxycinnamic acid; m.p. 210-212\textdegree (dec.) (lit. 2) 212\textdegree; yield 75\%; UV (ethanol): maxima at 300 and 327 mum; NMR (D\textsubscript{6}-acetone): \( \delta \) 6.93, 7.58 and 7.84 (3 singlets for 3H, aryl CH and olefinic H), 3.57 and 3.85 (6H, OMe).

Anal. calc. for C\textsubscript{20}H\textsubscript{21}NO\textsubscript{5}: 67.59\% C, 5.96\% H, 3.94\% N
found: 67.4 \% C, 6.1 \% H, 4.0 \% N.

\( \alpha \)-benzamido-2-isopropyl-4, 5-dimethoxycinnamic acid; m.p. 198-200\textdegree (dec.);
yield 75\%; UV (ethanol): maxima at 301 and 324 mum; NMR (D\textsubscript{6}-acetone): \( \delta \) 7.00, 7.30 and 7.92 (3 singlets for 3H, aryl CH and olefinic H), 3.58 and 3.86 (6H, OMe).

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Anal. calc. for C$_{21}$H$_{23}$NO$_5$ : 68.28% C, 6.28% H, 3.7% N
found : 68.5% C, 6.3% H, 3.7% N.

N-Benzoyl-3-(4, 5-dimethoxy-2-methylphenyl) alanine. The corresponding cinnamic acid (5g) was shaken with Raney-Ni in 250 ml of 0.25N NaOH for 2 hr at a pressure of 30 atm. of H$_2$.
The catalyst was filtered off. Acidification with HCl afforded the dihydrocinnamic acid, which was crystallized from ethanol. M. p. 202$^{9}$ (dec.) (lit. 12)209$^{9}$; yield 95%; UV (ethanol) maximum at 280 μμ.
Anal. calc. for C$_{19}$H$_{21}$NO$_5$ : 66.45% C, 6.16% H, 4.08% N
found : 66.2% C, 6.3% H, 4.3% N.
The following compound was prepared by the same route:

N-benzoyl-3-(2-ethyl-4, 5-dimethoxyphenyl) alanine; m. p. 186-188$^9$ (dec.);
yield 90%; UV (ethanol): maximum at 281 μμ.
Anal. calc. for C$_{26}$H$_{23}$NO$_5$ : 67.21% C, 6.49% H, 3.92% N
found : 66.8% C, 6.5% H, 3.8% N.

N-Benzoyl-3-(2-isopropyl-4, 5-dimethoxyphenyl) alanine. No complete hydrogenation could be effected on application of the method used above. By treating the mixture of the hydrogenated and nonhydrogenated compound in an alkaline solution with a Ni-Al alloy, as described by Nikiforov$^{10}$, a fully hydrogenated product was obtained. M. p. 177-180$^9$ (dec.);
yield 80-90%; UV (ethanol): maximum at 282 μμ.
Anal. calc. for C$_{21}$H$_{25}$NO$_5$ : 67.91% C, 6.79% H, 3.77% N
found : 67.7% C, 6.8% H, 3.7% N.

3-(4, 5-Dihydroxy-2-methylphenyl) alanine, (6-methyldopa). N-Benzoyl-3-(4, 5-dimethoxy-2-methylphenyl) alanine (5 g) was refluxed with 65 ml of freshly distilled 48% aqueous HBr under N$_2$. After cooling and dilution with water the solution was extracted with ether to remove the benzoic acid. Under vacuum the solution was evaporated to dryness as rapidly as possible at ca. 60$^0$. Next some water was added and the resulting solution was again evaporated to dryness.
The residual solid was dissolved in a minimum quantity of water, and by means of aqueous bicarbonate adjusted to pH 5-6.

After filtration, the solution was placed in a refrigerator for crystallization. The amino acid sometimes required a few days to separate. The colourless crystals were recrystallized from water containing a trace of SO$_2$. In most cases recovery was poor, however. It proved very difficult to remove the crystal water from the amino acid. The melting point was hard to determine because decomposition and carbonization occurred already at ca. 220$^0$.
M. p. ca. 255$^0$ (dec.) (lit. 12 269$^9$); yield 57%; NMR (D$_2$O+DCI); δ 2.19 (3H, CH$_3$), 3.15 (center of a multiplet, 2H, δ-CH$_2$), 4.21 (center mult., 1H, α-CH), 6.73 and 6.77 (singlets, 2H, aryl CH).

Anal. calc. for C$_{10}$H$_{13}$NO$_4$ : 56.86% C, 6.20% H, 6.63% N
found after drying at 100$^0$
(10$^{-3}$mm, P$_2$O$_5$) for 2 hr : 56.6% C, 6.3% H, 6.5% N.
The following two compounds were prepared in an analogous manner:

3-(2-ethyl-4, 5-dihydroxyphenyl) alanine, (6-ethyldopa); m. p. ca. 260$^0$
(dec.); yield 45%; NMR (D$_2$O+DCI); δ 1.16 (3H, CH$_3$), 2.55 (2H, ethyl CH$_2$), 3.18 (center mult., 2H, δ-CH$_2$), 4.23 (center mult., 1H, α-CH), 6.76 and 6.82 (singlets, 2H, aryl CH).
Anal. calc. for C$_{11}$H$_{15}$NO$_4$ : 58.65% C, 6.71% H, 6.22% N
found after drying at 100$^0$
(3mm, P$_2$O$_5$) for 24 hr : 58.8% C, 6.8% H, 6.1% N.
3-(4, 5-dihydroxy-2-isopropylphenyl) alanine, (6-isopropylidopa); m.p. ca 250° (dec.); yield 56%; NMR(D<sub>2</sub>O+DCI): δ 1.22 (6H, CH<sub>3</sub>), 2.75-3.75 (3H, β-CH<sub>2</sub> and isoprop. CH), 4.30 (center multit. 1H, α-CH). 6.88 and 7.02 (singlets, 2H, aryl CH).

Anal. calc. for C<sub>12</sub>H<sub>17</sub>NO<sub>4</sub> : 60.24% C, 7.16% H, 5.85% N
found after drying at 100° (10 °C, P<sub>2</sub>O<sub>5</sub>) for 4 hr : 60.0 % C, 7.1 % H, 5.9 % N.

2.3. ROUTE VIA ACETAMIDOMALONATE

Several authors<sup>8, 18, 19</sup> have succeeded in synthesizing alkylidopa derivatives by coupling a substituted benzyl chloride to the sodium compound of diethyl acetamidomalonate. The course of reaction is illustrated in scheme 3.

**SCHEME 3** Preparation of dopa derivatives via alkylation of acetamidomalonate

Chloromethylation reactions of veratrole lead to the formation of tetramethoxydiphenylmethane, tetramethoxyanthracene and macrocyclic condensation products<sup>20</sup>. Matsuno<sup>21</sup>, King<sup>22</sup>, and Clemo<sup>23</sup> were able to isolate the corresponding 5-chloromethyl compounds (see scheme 3) on chloromethylation of 4-ethyl- and 4-propylveratrole with formaldehyde-hydrogen chloride. In our investigations the application of this method to 4-methylveratrole afforded practically quantitative di(4, 5-dimethoxy-o-tolyl)methane.
Both Burger et al.\textsuperscript{13,18,19} and Edwards\textsuperscript{24} used chloromethylether for introducing a chloromethyl group into 3-alkylveratroles in 4-position. The last procedure enabled us to introduce a chloromethyl group into 4-ethyl- and 4-isopropylveratrole in 5-position. Recently Parulkar and Burger\textsuperscript{25} also synthesized 4-chloromethyl-5-methylveratrole by the same route. The yield they obtained was rather low (40\%) because the diarylmethane was formed at the same time.

An attempt, based on Clemo's method\textsuperscript{8} for the corresponding propyl derivative, was made to couple 4-chloromethyl-5-ethylveratrole to diethyl acetamidomalonate (schema 3) in ethanol with sodium ethoxide as the condensation agent. Under these conditions the chloromethyl product was found to react with sodium ethoxide while a benzyl-ethyl ether was formed. When the sodium compound of the acetamidomalonate was prepared in toluene by means of sodium hydride and then allowed to react with the benzyl halide, the formation of ether was prevented. The crude coupling product of the synthesis was further treated as indicated in scheme 3.

The desired N-acetyl-3-(2-ethyl-4, 5-dimethoxyphenyl)alanine (scheme 3, compound (A) for R=ethyl) could not be isolated by the working-up procedure applied here. When toluene\textsuperscript{26} or dioxane\textsuperscript{27} was used as a solvent for the malonate coupling, a quantity of a carboxylic acid was obtained, the IR spectrum of which showed the presence of the carboxyl group an amide-NH.

The equivalent weight, determined by titration with sodium hydroxide, however, suggested the presence of two 2-ethyl-4,5-dimethoxybenzyl groups in the molecule. The elemental analysis agreed well with the percentages calculated for \( \text{C}_{26}\text{H}_{35}\text{NO}_7 \), based on the following structure:

![Chemical structure](image)

Confirmation of this structure was obtained from the NMR spectrum (see experimental part).

The proton ratio was consistent with the above structural formula. The \( \text{CH}_3 \) protons in the acetamido group gave a singlet at 5.1.87, while the NH appeared at 56.20. The absence of any signal between 54.0 and 56.0 indicated the absence of \( \alpha \)-protons in the molecule. The magnetic nonequivalence of the two methylene protons in the \( \text{-CH}_2 \) -Ar groups gave rise to the appearance of an AB system for these protons (see Martin\textsuperscript{28} and Lansbury\textsuperscript{29}).
EXPERIMENTAL PART

Chloromethylation of 4-methylveratrole. Combination of 0.024 mole of 4-methylveratrole with 15 ml 40% aqueous formaldehyde and concentrated HCl at 0° furnished a white solid within a few min. This proved to be:

\[ \text{di(4, 5-dimethoxy-o-tolyl)methane; m.p. 122-123° (lit. 30) 124°).} \]

Anal. calc. for C\textsubscript{19}H\textsubscript{24}O\textsubscript{4}: 72.12% C, 7.65% H.
found: 72.0 % C, 7.6 % H.

4-Chloromethyl-5-ethylveratrole. Ethylveratrole (92.8 g) and 83.5 g of chloromethyl ether (n\textsubscript{D} = 1.3970) in 90 ml glacial acetic acid were stirred under N\textsubscript{2} for 6-10 hr. The temperature of the reaction mixture was maintained at 25-35°. The solution was poured onto ice and extracted with ether. Neutralization of the ethereal layer with aqueous bicarbonate, drying, evaporation of the ether and distillation gave an oil that solidified after some time. The compound was crystallized from petroleum ether (b.p. 28-40°) at ca. -30°. B.p. 125-140°/1.2 mm.; m.p. 42-44° (lit. 22) 40°; yield 56%.

Anal. calc. for C\textsubscript{11}H\textsubscript{15}ClO\textsubscript{2}: 16.51% Cl.
found: 16.1 % Cl.

An analogous procedure afforded:

4-chloromethyl-5-isopropylveratrole; b.p. 117-118°/10-1 mm.; n\textsubscript{D} = 1.5420;
yield 70%.

Anal. calc. for C\textsubscript{12}H\textsubscript{17}ClO\textsubscript{2}: 63.02% C, 7.48% H.
found: 63.0 % C, 7.4 % H.

Diethyl acetimidomalonate. Synthesized according to Snijder\textsuperscript{31} and Ghosh\textsuperscript{32}; m.p. 96-97.5° (lit. 32) 97°; yield ca. 50%.

Anal. calc. for C\textsubscript{8}H\textsubscript{15}NO\textsubscript{5}: 49.76% C, 6.96% H, 6.45% N.
found: 49.7 % C, 7.0 % H, 6.4 % N.

Reaction of 4-chloromethyl-5-ethylveratrole with acetimidomalonate. In 20 ml of anhydrous toluene 10 g (0.046 mole) of acetimidomalonate were converted into the Na compound by addition of an equimolar amount of NaH and subsequent heating for 1 hr. After 9.8 g of the chloromethyl compound in toluene had been added, the mixture was refluxed under N\textsubscript{2} for about 15 hr.

Following the procedure, described by Herr\textsuperscript{26}, an oil was obtained which did not crystallize. After heating the oil with alcoholic potassium hydroxide for 1 hr, washing the solution with ether and acidification, about 6 g of a solid could be isolated. The white compound was heated with 1N HCl and recrystallized several times form aqueous ethanol.

N-acetyl-3, 3-di(2-ethyl-4, 5-dimethoxyphenyl) alanine; m.p. 184-186°;
yield ca. 18%; IR(CHCl\textsubscript{3}): 3395 cm\textsuperscript{-1} (amide NH), ca. 3000 broad(carboxyl CH), 1720 (acid C=O) and 1670 (amide C=O); NMR (CDCl\textsubscript{3}): 51.10 (triplet, 6H, ethyl CH\textsubscript{2}), 1.87 (singlet, 3H, COCH\textsubscript{3}), 2.54 (quadruplet, 4H, ethyl CH\textsubscript{2}), 3.38 and 3.66 (AB system, J = 14 c/sec., benzyllic CH\textsubscript{2}), 3.68 and 3.78 (with benzyllic CH\textsubscript{2};16H, OMe), 6.20 (1H, NH), 6.60 and 6.63 (singlets, 4H, aryl CH), 10.40 (1H, COOH).

Anal. calc. for C\textsubscript{28}H\textsubscript{35}NO\textsubscript{7}: 65.87% C, 7.45% H, 2.96% N.
found: 66.3 % C, 7.3 % H, 2.9 % N.
LITERATURE

21. T. Matsuno, J. Pharm. Soc. Japan 64, 52 (1944); Chem. Abstr. 46, 126 (1952)
CHAPTER 3

PROBLEMS CONCERNING THE SYNTHESIS OF 6-TERN. BUTYLDOPA

3.1. INTRODUCTION

First an attempt was made to synthesize 6-tern. butyldopa by the route described for the isoproyl analogue in chapter 2.2. A Vilsmeier formylation of 4-tern. butylveratrole did not afford 6-tern. butylveratraldehyde. Using the previously described reaction conditions, 4-tern. butylveratrole was almost entirely recovered; by extending the reaction time and raising the bath temperature an oily mixture was obtained. The IR spectrum \(^1\) of this mixture indicated the presence of hydroxy groups and quinones. Under the latter conditions the methoxy groups were apparently unable to resist attack by phosphorus oxychloride.

An attempt to introduce the aldehydic group by means of ethyl orthoformate and aluminum chloride according to Gross \(^2\) provided no results either. It was then decided to introduce into the 5-position of 4-tern. butylveratrole another substituent which allowed further synthesis to the desired amino acid.

3.2. 4-TERN. BUTYL-5-NITROVERATROLE AND DERIVATIVES

Methylation of 4-tern. butylcatechol afforded the corresponding veratrole. The nitro compounds obtained from this veratrole according to the nitration procedures of Carpenter \(^3\) and Nishino \(^4\), as well as the hydrochlorides of the derived amines, are represented in the reaction scheme given below.
The 1, 2, 4, 5-substitution pattern of the formed dinitroveratrole was established by comparing the melting point with those reported for the four possible isomers. The same substitution pattern should be assigned to mononitro-tert. butylveratrole because more vigorous nitration of this compound gave rise to the above dinitroveratrole. This assignment was correct as appeared from the structure of the quinone, obtained in an attempt to convert 4-amino-5-tert. butylveratrole by diazotization and hydrolysis into the 2-tert. butyl-4, 5-dimethoxyphenol described by Thompson. The same quinone had already been isolated by Carpenter as a by-product in the nitration of the dimethyl ether of 4-tert. butylresorcinol.

The conversion of the amino compound into the quinone could not only be carried out with nitrous acid, but also with a dilute acid solution of ferric chloride, a reagent recently applied by Koshi in an analogous oxidation reaction. It is more obvious to assume that the quinone was formed directly by oxidation of the aminoveratrole under the influence of nitrous acid than that tert. butyl-dimethoxyphenol would be an intermediate in the conversion. In his experiments with an analogous phenol, Hewgill found that the formation of a quinone by oxidative demethylation takes place only if very specific oxidizing agents are used.
In order to establish unambiguously whether or not the tert.-butyl-methoxy-p-quinone is formed via the phenol, one would have to examine first the behaviour of the phenol under the experimental conditions of the diazotization.

The above experiments were based on the assumption that the synthesis of the desired amino acid might be possible via a Meerwein arylation (see e.g. Filler) and the synthesis of α-methyl-dopa).

The route offered few prospects, because the method of diazotization in aqueous solution could not be applied to 4-amino-5-tert.-butylveratrole. On the other hand, the synthesis of 6-tert.-butylverataldehyde (see chapter 3.3) permitted the route via the azlactone.

Therefore, it was decided to employ the latter route in our further investigations into the possibilities to synthesize 6-tert.-butyl-dopa.

**EXPERIMENTAL PART**

4-tert.-Butylveratrole. Synthesis as described for 4-isopropylveratrole in chapter 2.2. B.p. 114-118°; crystallization from petroleum ether (b.p. 28-40°), m.p. 34-35° (lit. 36-37°); yield ca. 85%.

4-tert.-Butyl-5-nitroveratrole. Prepared by nitration of 4-tert.-butylveratrole according to Nishino; crystallization from aqueous methanol; m.p. 49-51°; yield 68%.

Anal. calc. for C_{12}H_{17}NO_4: 5.8% N
found: 5.9% N.

Nitration with fuming nitric acid. Nitration according to Carpenter with fuming HNO_3 in acetic acid and acetic anhydride of 4-tert.-butylveratrole and 4-tert.-butyl-5-nitroveratrole gave 4, 5-dinitroveratrole. M.p. 130-131° (lit. 130-131°); yield ca. 70%.

Anal. calc. for C_{16}H_{8}N_2O_6: 12.28% N
found: 12.3% N.
4, 5-Diaminoveratrole dihydrochloride. This compound was synthesized from the dinitro compound by reduction, using Raney-Ni and H₂ (3 atm.) in ethyl acetate for 4 hr. The dihydrochloride was obtained by passing HCl into the solution after the catalyst had been filtered off. The salt was found to oxidize when exposed to air; on standing, excluded from the air, the pale product assumes a violet colour.

When heated above 200° the compound decomposed.

Mol. weight calc. for C₁₀H₁₄Cl₂N₂O₂ : M = 241
found by titration with NaOH : M = 243.

4-Amino-5-tert. butylveratrole hydrochloride. Synthesized by reduction of the nitro compound with H₂ (30 atm.) under the influence of Raney-Ni in ethyl acetate at 50° for 3 hr. After the catalyst had been filtered off, the solvent was evaporated. Next the remaining mass was treated with ether - HCl. The resulting brown solid was dissolved in hot acetone-chloroform and precipitated by the addition of ether. The crude, colourless hydrochloride (yield: ca. 70%) was crystallized from chloroform-petroleum ether. The free amine immediately oxidized when exposed to air.

Found by titration with NaOH : M = 246 (calc.:M= 246)
Anal. calc. for C₁₂H₂₀ClNO₂ : 58.64 % C, 8.20 % H, 5.70 % N, 14.43 % Cl
found : 58.4 % C, 8.1 % H, 5.9 % N, 14.6 % Cl.

Diazotization of 4-amino-5-tert. butylveratrole hydrochloride. After dissolving 0.8 mmole of the hydrochloride in 1.5 ml of 2N H₂SO₄, 0.75 mmole of NaNO₂ in 1 ml of water was added at 0°.

On exposure to the air at room temperature the green colour of the solution changed to yellow and 2-tert. butyl-5-methoxy-p-benzoquinone separated as a yellow solid (yield ca. 65%). The same quinone was isolated by pouring the solution of the "diazonium salt" into hot aqueous CuSO₄ (see Thompson[6]). M.p. 160-162° (lit. 3,13) 162-163°, 160°; IR (0.5% in KBr): ca. 1640 and 1670 cm⁻¹ (C=O).

Anal. calc. for C₁₁H₁₄O₃ : 68.02 % C, 7.27 % H
found : 68.1 % C, 7.1 % H.

3.3. 4-BROMO-5-TERT. BUTYLVERATROLE AND DERIVATIVES

The smooth introduction of a nitro group into the 5-position in 4-tert. butylveratrole by careful nitration prompted us to investigate the bromination of this compound as well.

Brominations of 4-tert. butylcatechol and the derived monomethyl ethers have been reported by Bell[8] and Buu-Hoi[14,15], but in neither case could the position of the bromine atom be established with certainty.

Bromination of 4-tert. butylveratrole in carbon tetrachloride at a low temperature afforded the desired 5-bromo derivative in good yield.

The 1, 2, 4, 5-substitution pattern of the aryl bromide could be unambiguously established by means of the NMR spectra of the bromide and its derivatives, which were obtained via Grignard reactions, as described below.
The bromide was almost entirely converted into the Grignard compound by applying an entrainment reaction and using tetrahydrofuran (THF) as the solvent (see Schmiechen\textsuperscript{16}). After carbon dioxide had been passed through the solution, working-up the reaction mixture gave 6-tert. butylveratric acid (yield ca. 50\%) in addition to 4-tert. butylveratrole (yield ca. 50\%). The Grignard compound was found to be extremely sensitive to water so that fully anhydrous conditions were required.

The synthesis of 6-tert. butylveratraldehyde via the reaction of the Grignard reagent with ethyl orthoformate\textsuperscript{17} was not successful. The use of N-methylformanilide, as reported by Feugeas\textsuperscript{18} for the preparation of piperonal, did give a favourable result.

The purpose of synthesizing 4-tert. butyl-5-methylveratrole was to obtain a link between our work and literature data. Pospíšil\textsuperscript{19} prepared in 1965 the same dialkylveratrole by butylation of 4-methylveratrole.

The melting point and refractive index of the tert. butyl-methylveratrole obtained were in reasonable agreement with the values found by Pospíšil (see experimental part).

NMR spectral data of bromo-tert. butylveratrole and its derivatives are shown in table 1. According to literature\textsuperscript{20} the coupling constants of aromatic protons have, depending on their relative positions, the following order of magnitude:

<table>
<thead>
<tr>
<th>Position</th>
<th>Coupling Constant (c/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ortho</td>
<td>7-9</td>
</tr>
<tr>
<td>meta</td>
<td>1-3</td>
</tr>
<tr>
<td>para</td>
<td>ca 0.3</td>
</tr>
</tbody>
</table>


In the NMR spectra of the first four compounds in table 1 no coupling could be
observed between the separated signals of the two aryl protons \(J_{AB} \approx 0\). It was
concluded that the two aryl protons in these molecules did certainly not occupy
an ortho position with reference to each other and probably not a meta position
either. Conclusive evidence that a meta position was excluded completely, was
afforded by a comparison of the NMR spectrum of the produced tert. butyl-methyl-
veratrole with the spectra of the two isomers in which the aryl protons do oc-
cupy a meta position in relation to each other.

\[\text{5-tert-butyl-3-methyl veratrole} \quad \text{3-tert-butyl-5-methyl veratrole}\]

The two meta-dialkylveratroles were prepared by methylation of the correspond-
ing catechols.

<table>
<thead>
<tr>
<th>Veratroles</th>
<th>Chemical shifts(^a)(5, p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tB</td>
</tr>
<tr>
<td>4-bromo-5-tB</td>
<td>1.47</td>
</tr>
<tr>
<td>4-tB-5-CHO(^b)</td>
<td>1.49</td>
</tr>
<tr>
<td>4-tB-5-COOH(^c)</td>
<td>1.48</td>
</tr>
<tr>
<td>4-tB-5-Me</td>
<td>1.35</td>
</tr>
<tr>
<td>5-tB-3-Me</td>
<td>1.28</td>
</tr>
<tr>
<td>3-tB-5-Me</td>
<td>1.32</td>
</tr>
<tr>
<td>3, 5-di-tB</td>
<td>1.31;1.39</td>
</tr>
<tr>
<td>bromo-3, 5-di-tB</td>
<td>1.36;1.50</td>
</tr>
<tr>
<td>nitro-3, 5-di-tB</td>
<td>1.37;1.44</td>
</tr>
</tbody>
</table>

\(^a\)Solvent CDCl\(_3\); standard TMS; temperature ca 380. \(^b\)516.51 (CHO). \(^c\)5 12.10 (COOH).
\(^d\)The aryl protons appeared as singlets, except when the coupling constant is given in the table.
ADDENDUM: Bromination of 3,5-ditert. butylveratrole.

The favourable results obtained in the bromination of tert. butylveratrole raised the question whether it would be possible, following the same procedure, to introduce a bromine atom into the 4-position in 3,5-ditert. butylveratrole, as the first step in the synthesis of 2,6-ditert. butyldopa. Bromination of 3,5-ditert. butylveratrole in carbon tetrachloride or glacial acetic acid at varying temperatures only afforded the unchanged starting material. Betts's method\textsuperscript{21} for bromination of 1,3,5-tritert. butylbenzene with a mixture of bromine, nitric acid, silver nitrate and acetic acid was adopted in our bromination of 3,5-ditert. butylveratrole. GLC of the reaction mixture pointed to the presence of at least six components in addition to some starting material. Some insight into the structures of these components was obtained by collecting various fractions, followed by purification and determination of IR and NMR spectra (see table 1). Only two components appeared to be a compound in which the original tert. butyl and methoxy groups were still present.

![Chemical structure](image)

The observation that on bromination and nitration of monotert. butylveratrole substitution was exclusively para to the methoxy groups (see chapter 3.2 and 3.3), suggests that in the substituted ditert. butylveratroles obtained the substituent has entered the position between the tert. butyl groups. However, no conclusive evidence for this assumption has been found yet.

Heating bromo-3,5-ditert. butylveratrole with magnesium and ethylene dibromide in THF, followed by addition of water, gave a reaction product which, as appeared from its NMR spectrum, consisted of ca. 50\% of unreacted bromo compound and ca. 50\% of 3,5-ditert. butylveratrole. It follows that there is no doubt as to the positions of the tert. butyl and methoxy groups in the molecule.

**EXPERIMENTAL PART**

NMR spectral data are given in table 1.

4-Bromo-5-tert. butylveratrole. To a solution of 20 g tert. butylveratrole in 35 ml of anhydrous CCl₄, cooled to -20°, was added a solution of 16.8 g Br₂ in 25 ml CCl₄ at such a rate that the temperature in the reaction flask did not exceed -10°. After addition of an aqueous
solution of sodium bisulphite the organic layer was separated, washed and dried. The crude bromide, obtained by distillation, was crystallized form methanol-water. B.p. 157-159°/12 mm; 65-66°; yield ca. 80%; GLC and TLC analyses showed a single compound.

Anal. calc. for C_{12}H_{17}BrO_{6}: 52.76% C, 6.27% H, 29.26% Br
found : 52.9 % C, 6.4 % H, 29.5 % Br.

6-tert. Butylveratric acid. To 0.05 mole of magnesium in anhydrous THF were slowly added 0.015 mole of 4-bromo-5-tert. butylveratrole together with 0.005 mole of ethylene dibromide in such an amount of THF that a total volume of 15-20 ml was reached. The THF was made anhydrous by distillation from Na, followed by distillation from LiAlH₄.

The Grignard reaction was carried out under N₂, using glassware dried at 110° for a few hours. After addition of the bromides, the mixture obtained was refluxed for some hours. Next CO₂ was passed through at a bath temperature of about 70° for 12 hr, after which the mixture was poured into dilute HCl. The ether extract of the acid solution was extracted with 2N NaOH; acidification of the basic extract gave the crude veratric acid in a yield of about 50%. The acid was crystallized from petroleum ether. The remaining ether extract was washed, dried and evaporated to give a 50% yield of 4-tert. butylveratrole, which was identified by means of the IR spectrum.

6-tert. Butylveratraldehyde. To the THF solution of the Grignard reagent (see above) was slowly added 0.03 mole of N-methylformanilide (b.p. 121-123°/15 mm). The volume of THF was increased to 30 ml and then stirred under N₂ at room temperature for 18 hr.

The mixture was poured into dilute H₂SO₄ at 0°, followed by stirring at room temperature for 1 hr. By extraction with benzene, followed by distillation, the crude aldehyde could be isolated in a yield of ca. 50% as a light-yellow oil. An attempt to convert the aldehyde into a bisulphite addition compound failed.

B.p. 97-103°/10⁻³ mm; nD₂⁰: 1.5550; IR (5% in CHCl₃): 1665 cm⁻¹ (C=O).
Anal. semicarbazone (m.p. 155-158°)
calc. for C_{14}H_{21}N₃O₃: 60.20% C, 7.58% H, 15.04% N
found : 59.7 % C, 7.6 % H, 14.7 % N.

4-tert. Butyl-5-methylveratrole. A solution of 0.028 mole of dimethylsulfate in 20 ml of THF was added to the THF solution of the Grignard reagent (see above) at a bath temperature of 70° over a period of 1 hr. Next the solution, in which a precipitate had formed, was stirred under heating for 19 hr. Addition of 30 ml of 0.25N HCl gave rise to a two-layer system, which was extracted with toluene several times.

After washing and drying, the toluene extract was distilled to give 2.4 g of an oil (b.p. 125-135°/10 mm). GLC (column 20% SE 30, temp. 230°) showed that the oil consisted of 35% of 4-tert. butylveratrole and 65% of 4-tert. butyl-5-methylveratrole.

By collecting the latter fraction from the column, some methyl derivative could be isolated in a reasonably pure state.

M.p. 5-8° (lit. 19° 5.5-9.5°) ; νC=O: 15212 (lit. 19° 1,5196).
Anal. calc. for C_{13}H_{26}O₂ : 74.36% C, 9.68% H
found : 74.6 % C, 9.5 % H.

3-tert. Butyl-5-methylveratrole. Synthesis achieved by methylation of the corresponding catechol as described in chapter 2.2 for the preparation of 4-isopropylveratrole. B.p. 60-70°/4 mm; nD₂⁰: 1.5079.

Anal. calc. for C_{13}H_{26}O₂ : 74.96% C, 9.68% H
found : 74.9 % C, 9.5 % H.
5-tert. Butyl-3-methylveratrole. Synthesis from the catechol in the manner described in chapter 2.2. B. p. 125-128°/11 mm; n\textsuperscript{20}D: 1.5652.

Anal. calc. for C\textsubscript{13}H\textsubscript{20}O\textsubscript{2}: 74.96% C, 9.68% H

found: 74.9 % C, 9.5 % H.

3,5-Ditert. butylveratrole. Synthesized from the catechol as described in chapter 2.2. The yields were low as a result of incomplete methylation. B. p. 90-95°/4 mm; crystallization from ethanol-water; m. p. 50.5-55°; NMR: see table 1.

Bromination of 3,5-ditert. butylveratrole. To a mixture of 10 g of ditert. butylveratrole in 120 ml of glacial acetic acid, 2.5 ml of Br\textsubscript{2} in 10 ml of glacial acetic acid and 20 ml of HNO\textsubscript{3} (sp. gr. 1.2) in 40 ml of water were added 6.8 g of AgNO\textsubscript{3} in 20 ml of water over a period of 1 hr. After addition of 80 ml of glacial acetic acid, the mixture was stirred at 36° for 6 hr, and then poured into 60 ml of water. Next, the AgBr was filtered off and washed with glacial acetic acid.

The neutralized filtrate (pH 7) was extracted with ether and the AgBr was washed with chloroform; the solutions were combined and the solvents evaporated to give the reaction products, which were isolated in a more or less pure state by distillation under reduced pressure and GLC (column 20% SE 30, temperature ca. 150°). The fractions containing compounds with two tert. butyl groups in the molecule were purified further by crystallization.

Bromo-3,5-ditert. butylveratrole; m. p. 90-92°; yield ca. 20%; NMR: see table 1.

Anal. calc. for C\textsubscript{16}H\textsubscript{25}BrO\textsubscript{2}: 58.35% C, 7.62% H, 24.23% Br.

found: 58.8 % C, 7.6 % H, 24.0 % Br.

Nitro-3,5-ditert. butylveratrole; m. p. 130-131°; yield ca. 20%; NMR: see table 1.

Anal. calc. for C\textsubscript{16}H\textsubscript{25}NO\textsubscript{4}: 65.05% C, 8.53% H, 4.74% N

found: 65.2 % C, 8.7 % H, 4.9 % N.

3.4. SYNTHESIS OF THE AMINO ACID VIA THE AZLACTONE

Since the synthesis of 6-tert. butylveratrionaldehyde (chapter 3.3) was successful, it seemed that the reactions shown in scheme 2 (chapter 2.2), with the aldehyde as the starting material, would be a possible route to 6-tert. butyl dopa. The yields of azlactone afforded by condensation of the aldehyde with hippuric acid were, however, disappointing.

![Diagram of synthesis reaction]

An attempt was made to find another synthetic route in which 4-bromo-5-tert. butylveratrole (chapter 3.3) could be used more efficiently.

Such a route was found in the work of Behringer\textsuperscript{23}), who synthesized azlactones by using the reactivity of the methylene carbon atom in 4-chloromethylene-2-phenyl-2-oxazolin-5-one toward nucleophiles.
According to Behringer, organometallic compounds of moderate reactivity, such as 3-indoly1- and 2-pyrrolylmagnesium bromide and arylcadmium compounds, were successfully employed as nucleophilic reagents. Aryl and alkyl magnesium bromides presented difficulties as a result of the competitive attack on the carbon atom of the carbonyl group. In general azlactones offer two possibilities with regard to the addition of a Grignard reagent followed by an elimination step.

Which reaction will occur depends on several factors: for \( Y = \text{halogen or alkoxy} \), 1,4-addition takes place with Grignard reagents of moderate reactivity (Behringer\(^23\)) and Hiraoka\(^24\)); for \( Y = \text{aryl} \), 1,4-addition compounds are formed on condition that \( R = \text{alkyl} \) (Horner\(^25\)); for \( Y \) and \( R = \text{aryl} \), 1,2-addition is observed (Awad\(^26\) and Pourrat\(^27\)). The influence of catalysts (cuprous chloride) on the reaction and the influence of the geometric structure of the azlactone concerned (see also chapter 4) have been examined by Filler\(^28\).

The good results of Behringer's azlactone synthesis\(^23\) induced us to allow the Grignard compound of 4-bromo-5-tert, butylveratrole to react with the above chloromethylene-oxazolinone which had been prepared from hippuric acid as described in literature\(^23\).
Experiments under varying conditions were performed to bring about the addition of the Grignard compound—whether or not via the cadmium compound—to the vinylogous carboxylic acid derivatives.

\[
\begin{align*}
&\text{MeO} \quad \text{MeO} \\
&\text{Br} \quad \text{Mg}_{2}\text{THF} \quad \text{MeO} \\
&\text{YCH} = \text{N} \quad \text{Ph} \quad \text{YCH} = \text{N} \quad \text{Ph}
\end{align*}
\]

A) \( Y=\text{Cl} \)
B) \( Y=\text{OEt} \)

A) When \( Y=\text{Cl} \), the azlactone was obtained in a yield of ca. 30% by allowing the chloromethylene compound and the Grignard compound to react in THF at -40\(^{\circ}\)C. On analysis of the reaction mixture not only the veratrylidene-oxazolinone was found but also 30-40% of tert. butyveratrole, formed from the Grignard reagent which had remained unchanged during the reaction. Raising the temperature lowered the production of azlactone and increased the yield of black tarry by-products.

B) The replacement of the vinylogous acid chloride with the vinylogous ester (\( Y=\text{OEt}:4\)-ethoxymethylene-2-phenyl-2-oxazolin-5-one) afforded an interesting reaction. Behringer\(^{23}\) has reported on only one attempt to add a Grignard compound to ethoxymethylene-oxazolinone, the result being a reaction product with an unidentified structure. When the reaction shown in the above scheme was performed in THF at -40\(^{\circ}\) and the reaction mixture worked up by pouring it into an aqueous solution of ammonium chloride, the tert. butyveratrylidene-oxazolinone (yield: 15%) and tert. butyveratrole (yield: 40%) and also the vinylogous amide (yield: 30%), formed from the unreacted ethoxymethylene compound, were isolated.

\[
\begin{align*}
&\text{EtOCH} = \text{N} \\
&\text{Ph} \quad \text{NH}_{4}\text{Cl} \quad \text{H}_{2}\text{NCH} = \text{N} \quad \text{Ph}
\end{align*}
\]

Strukov\(^{29}\) performed this conversion from "ester" into "amide" using alcoholic ammonia.

In our experiments with the ethoxymethylene compound, the amount of tarry products was markedly lower than when the chloromethylene compound was used.
The reactions were carried out under varying experimental conditions, the most favourable results being obtained by the procedure (see also experimental part) in which the Grignard compound in THF was added slowly under nitrogen to a vigorously stirred solution of the ethoxymethylene compound in a large volume of ether. This procedure gave the desired azlactone and a colourless solid which was identified as the ethyl ester of \( \alpha \)-benzamido-2-tert. butyl-4, 5-dimethoxycinnamic acid by IR and NMR spectroscopy.

![Chemical structure](image)

The azlactone produced in the addition reactions was identical to that obtained in low yield by the Erlenmeyer-Plöchl synthesis. Alkaline hydrolysis of the latter azlactone afforded the corresponding cinnamic acid which, when allowed to react with ethanol under the influence of sulphuric acid, gave an ethyl ester identical to the ester in the reaction mixture of the above Grignard addition to ethoxymethylene-oxazolinone.

These conversions indicate that the tert. butyl-substituted azlactone and ester, obtained by the addition reaction, have the same geometric configuration (see further chapter 4)

Since it was possible to convert both the azlactone and the ester into the \( \alpha \)-benzamidocinnamic acid required by treatment with sodium hydroxide, followed by acidification, these two compounds did not have to be separated for preparative purposes (see scheme 4, chapter 3.6). The yield of substituted cinnamic acid, calculated with reference to the bromide, amounted to 45%.

At first the origin of the ethyl group in the ester was unknow because the working-up procedure had included operations, such as crystallization, in which ethanol had been used. On repetition of the experiment all ethanol was replaced by methanol but again the ethyl ester was produced. It was therefore concluded that the ring was opened and the ester formed by a probably intermolecular- attack of the leaving group.
When a solution of 4-(4, 5-dimethoxy-2-methylbenzylidene)-2-phenyl-2-oxazolin-5-one in ether was treated with a solution of ethoxymagnesium bromide in THF, the ethyl ester of the corresponding α-benzamidocinnamic acid was obtained, a result which supports the intermolecular route proposed above.

\[
\begin{align*}
\text{EtMgBr} + \text{EtOH} & \overset{\text{THF}}{\rightarrow} \text{EtOMgBr} \\
& \overset{\text{b) } H^+}{\longrightarrow} \text{ArCH=COOEt}
\end{align*}
\]

\[(\text{Ar} = 4, 5\text{-dimethoxy-2-methylphenyl}; \text{the yield of the ester was about } 55\%)\]

Shortly after our findings Hiraoka\(^{24}\) reported two examples of the formation of the ethyl ester of an acrylic acid derivative by addition of a Grignard reagent to ethoxymethylene-oxazolinone.

\[
\begin{align*}
\text{R} & \equiv \text{C} \equiv \text{C-MgBr} \\
\rightarrow & \text{R} \equiv \text{C} \equiv \text{CH=COOEt} \\
\text{EtOCH} & \equiv \text{N} \equiv \text{N-Ph}
\end{align*}
\]

\(\text{R} = \text{phenyl and } n\text{-butyl}\)

In his experiments the esters were obtained in satisfactory yields but no mention is made of any corresponding azlactones that might still be present.

In order to continue the synthesis of the amino acid, α-benzamido-2-tert.butyl-4, 5-dimethoxycinnamic acid was converted into the dihydro product by reduction with a nickel-aluminum alloy (see scheme 4, chapter 3.6).
EXPERIMENTAL PART

4-(2-tert. Butyl-4, 5-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-one. The synthesis was similar to that described in chapter 2.2 for 2-alkyl-4, 5-dimethoxybenzylidene-oxazolinones from aldehydes and hippuric acid. Yellow solid; m.p. 160-161°C; yield 5-12%; IR (CHCl₃): 1785 cm⁻¹ (C=O) and 1650 (C=N); NMR see chapter 4.
Anal. calc. for C₂₂H₂₈NO₄: 72.31% C, 6.34% H, 3.83% N
found: 72.5% C, 6.4% H, 3.7% N.

4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one. Synthesized according to Behringer. Crystallization from ethanol gave flesh-coloured crystals; m.p. 96-97.5°C (lit. 97-98°C).

4-Chloromethylene-2-phenyl-2-oxazolin-5-one. This product was synthesized according to Behringer from the corresponding ethoxymethylene compound. Two different melting points are reported in the literature, viz. 135-137°C and 126-127.8°C. We found both melting points depending on the method of determination. A measurement in a capillary tube showed a melting point of 127-128°C whereas the melting point microscope revealed a higher value, viz. 135°C under phenomena of sublimation. From the yellow chloromethylene product, crystallized from ethylene dichloride, a colourless product was obtained by sublimation, followed by crystallization. The elemental analysis of both the yellow and the colourless compound yielded correct values. The two compounds are presumably not geometric isomers in view of the full identity of their UV spectra. The work of Cornforth shows that dimorphism may occasionally occur in heteromethylene-oxazolinones.
Anal. calc. for C₁₀H₁₀ClNO₂: 57.85% C, 2.91% H, 17.08% Cl, 6.75% N
found: 57.9% C, 2.9% H, 17.2% Cl, 6.6% N.

Grignard reagent plus ethoxymethylene-oxazolinone at -40°C in THF. 4-Bromo-5-tert. butylveratrole (0.006 mole) was converted into the Grignard compound as described in chapter 3.3, exp. part. At -40°C, 0.006 mole of the ethoxymethylene compound in THF was added. The resultant mixture was stirred at the same temperature for 1-1.5 hr and next poured into aqueous NH₄Cl, which was subsequently extracted with a large volume of ether. From the ethereal layer three compounds were isolated: the above tert. butyl-dimethoxymethylene-oxazolinone (yield: 15%), 4-tert. butylveratrole (yield: 40%) and 4-amino-methylene-2-phenyl-2-oxazolin-5-one.
M.p. 212-215°C (lit. 209°C); yield ca. 30%; U.V. (31) (ethanol): maximum at 343 mλ (ε 28000).
Anal. calc. for C₁₀H₈N₂O₂: 63.82% C, 4.29% H
found: 63.4% C, 4.5% H.

α-Benzamido-2-tert. butyl-4, 5-dimethoxycinnamic acid. In the manner described in chapter 3.3, 15.3 g (0.086 mole) of 4-bromo-5-tert. butylveratrole were converted into the Grignard compound by means of 5 g (0.027 mole) of ethylene dibromide and 3 to 4 g of magnesium in 85 ml of anhydrous THF.

The Bluish green solution was transferred to a dropping funnel in such a way that the excess magnesium was left behind.

For this purpose the N₂ pressure in the original three-necked flask was increased with the result that the THF solution was forced form the flask into the well-dried dropping funnel via a glass tube that reached to the bottom of the flask.

The Grignard solution was then added over a period of 15 min to a vigorously stirred solution of 15.0 g (0.06 mole) of ethoxymethylene-oxazolinone in about 500 ml of anhydrous ether. The mixture obtained was refluxed for 1 hr. The set-up of the apparatus was so chosen that the air was excluded from the Grignard solution during manipulations.

The red, half-solid mass in the yellow ethereal solution was worked-up by pouring it into water, followed by acidification to litmus with 4N acetic acid, separation of the organic layer, extraction of the aqueous layer with toluene-chloroform and washing and drying of the combined
ether-toluene-chloroform fractions. The solvents were removed to give a brownish red oil. (IR (CHCl₃); several strong bands in the range of 1600-1800 cm⁻¹ (C=O/C=N))

The oil was worked up in two manners for identification (A) and preparative purposes (B).

(A). The oil was dissolved in hot ethanol/methanol, cooled and crystallized to give tert. butyl-dimethoxybenzylidene-oxazolinone (yield: about 12%). After the azlactone had been filtered off, the alcoholic solution was evaporated and the residue stirred with 75 ml of 3% aqueous NaOH and about 100 ml of ethanol/methanol. After some time a solid separated, which was identified as the ethyl ester of the above cinnamic acid after crystallization from chloroform-petroleum ether. Physical data of the ester are given in a subsequent part of this experimental section.

(B). Treatment of the oil with 125 ml of 5% aqueous NaOH and 125 ml of ethanol at 60-80° for 1 hr, cooling, evaporation of the alcohol, washing with ether, filtration and acidification gave α-benzamido-2-tert. butyl-4, 5-dimethoxycinnamic acid. This product was crystallized from ethanol-water. M.p. 188-192° (dec.); yield 45%; UV (ethanol): maxima at 303 and 316 mμ; NMR (D₂-acetone): δ 7.11, 7.23 and 8.25 (3 singlets for 3 H, aryl CH and olefinic H), 3.62 and 3.85 (6H, OMe), 1.44 (9H, tert. butyl CH₃), for details see chapter 4.

Anal. calc. for C₇₂H₈₀NO₅ : 68.90% C, 6.57% H, 3.65% N
found : 68.9 % C, 6.7 % H, 3.7 % N.

Ethyl α-benzamido-2-tert. butyl-4, 5-dimethoxycinnamate. The heating of the corresponding cinnamic acid in ethanol with a few drops of conc. H₂SO₄ at 80-85° for 15 hr afforded the ethyl ester.

M.p. 147-149°; IR (CHCl₃): 3430 cm⁻¹ (NH), 1715 (C=O ester) and 1675 (C=O benzamido); NMR spectral data are given in chapter 4.

Anal. calc. for C₂₄H₂₈NO₅ : 70.04% C, 7.10% H, 3.40% N
found : 70.2 % C, 7.1 % H, 3.5 % N.

Ethyl α-benzamido-4, 5-dimethoxy-2-methylcinnamate. Ethoxymagnesium bromide was prepared from 0.01 mole of ethyl bromide and 0.01 mole of magnesium in THF, followed by the addition of 0.01 mole of ethanol. As described above, the resultant mixture was forced under N₂ into an ethereal solution of 0.01 mole of 4-(4, 5-dimethoxy-2-methylbenzylidene)-2-phenyl-2-oxazolin-5-one (see chapter 2.2) and next worked-up to give a mixture of the azlactone and the ethyl ester of α-benzamido-4, 5-dimethoxy-2-methylcinnamic acid. On saponification of the ester the same cinnamic acid as that mentioned in chapter 2.2 was obtained. Crystallization of the azlactone ester mixture afforded the ester in 55% yield, M.p. 133-135°; NMR: see chapter 4.

Anal. calc. for C₂₁H₂₃NO₅ : 68.28% C, 6.28% H, 3.79% N
found : 68.3 % C, 6.2 % H, 3.7 % N.

N-Benzoyl-3-(2-tert. butyl-4, 5-dimethoxyphenyl) alanine. Synthesized by hydrogenation with a Ni-Al alloy of the related α-benzamidocinnamic acid (see chapter 2.2 for the corresponding isopropyl derivative). M.p. 185-186° (dec.); yield 80%; UV (ethanol): maximum at 230 mμ.

Anal. calc. for C₂₂H₂₇NO₅ : 68.55% C, 7.06% H, 3.62% N
found : 68.4 % C, 7.1 % H, 3.5 % N.
3.5. REMOVAL OF THE PROTECTING GROUPS

On the analogy of the experiments in chapter 2.2 an attempt was made to remove the protecting groups in N-benzoyl-3-(2 tert. butyl-4, 5-dimethoxyphenyl) alanine (see chapter 3.4) with concentrated hydrobromic acid. The only amino acid that could be isolated after the reaction was completed, was (unsubstituted) dopa; the tert. butyl group had been removed from the molecule together with the protecting groups. The use of concentrated hydrochloric acid gave a similar result. Even if experimental conditions allowed only the benzoyl group to leave and ensured that the ether bonds remained intact, the tert. butyl group was split off.

This reaction course was not entirely unexpected. In 1963 Anderson\textsuperscript{32} had found the tert. butyl group to split off when tert. butylphenols were boiled with hydrobromic acid; however, the use of concentrated hydrochloric acid resulted in a much smaller loss of tert. butyl groups. The loss of tert. butyl substituents, due to the influence of concentrated hydrohalogen acids, prevented Nikiforov\textsuperscript{33} in 1962 from succeeding in his attempts to synthesize 3,5-ditert. butyltyrosine.

In the same year Cohen\textsuperscript{34} did obtain 3,5-ditert. butyltyrosine, though in a low yield, by carrying out the debenzoylation in an alkaline medium. The inconvenient side reaction in the removal of the protecting groups in an acid
medium led us to explore the possibility of splitting off the N-benzoyl group and the O-methyl groups in succession in various reaction steps. It also appeared possible to remove the benzoyl group in a strongly alkaline medium\textsuperscript{34, 35, 36}. The demethylation of the aryl methyl ethers required a reagent that left the remainder of the molecule intact. As in the case of boron halogen reagents the reaction conditions were extremely mild compared to those under which aromatic ethers were cleaved by means of other Lewis acids\textsuperscript{37}, our choice fell on boron tribromide.

McOmie\textsuperscript{38} reported successful demethylation of some twenty different aryl methyl ethers, including substituted veratroles, with this reagent at -80\textdegree and Pages\textsuperscript{39} accomplished the cleavage of the methyl ether in a substituted 4-methoxybenzylidene-oxazolinone without any undesired side reactions.

In order to establish whether boron tribromide caused no undesired transalkylation reactions\textsuperscript{40} in a tert. butyl-substituted veratrole, 4-tert. butyl-5-methyl-veratrole (see chapter 3.3) was treated with this reagent under the conditions described by McOmie\textsuperscript{38}. The catechol to be expected should be identical to one of the reaction products of the butylation of 4-methylcatechol, as performed by Pospíšil\textsuperscript{19}.

![Chemical reaction diagram]

The NMR spectrum of the tert. butyl-methylcatechol obtained in good yield, showed unambiguously the correctness of the structure given in the scheme. Table 2 lists NMR spectral data on this compound, and on a number of available isomers for comparative purposes. After crystallization the melting point of 4-tert. butyl-5-methylcatechol agreed with the value reported by Pospíšil (see experimental part).


<table>
<thead>
<tr>
<th>Catechols</th>
<th>Chemical shifts (^1) ((\delta), p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(tB)</td>
</tr>
<tr>
<td>4-tB-5-Me</td>
<td>1.29</td>
</tr>
<tr>
<td>5-tB-3-Me</td>
<td>1.21</td>
</tr>
<tr>
<td>3-tB-5-Me</td>
<td>1.38</td>
</tr>
<tr>
<td>3-tB-6-Me</td>
<td>1.38</td>
</tr>
</tbody>
</table>

\(^1\) Solvent \(CDCl_3\); standard TMS; temperature ca. 38\(^\circ\). 

The synthesis of 6-tert. butyldopa with the above 3-aryl-N-benzoylalanine as the starting material, presented some problems when demethylation under the influence of boron tribromide and debenzoylation in a strongly alkaline medium were performed in succession.

When first demethylation was carried out, the dihydroxy compound, which is readily oxidized in an alkaline medium, offered experimental difficulties in the next step, debenzoylation, which required the heating of the compound with concentrated aqueous sodium- or barium hydroxide for about five days. When first the benzoyl group was removed, the demethylation procedure had to be applied to an amino acid hard to isolate and insoluble in solvents such as methylene chloride, described\(^38\) for the reaction with boron tribromide.

The latter problem could be solved by attaching a new protecting group to the nitrogen atom after removal of the benzoyl group, thus facilitating isolation of the amino acid and resulting in a greater solubility in organic solvents. It was, however, necessary for the new protecting group to be resistant to boron tribromide and besides, removal of the group under mild conditions had to be possible. The phthaloyl group has been found to meet all these requirements perfectly\(^41\).

After the cleavage of the benzoyl group in N-benzoyl-3-(2-tert. butyl-4, 5-dimethoxyphenyl)alanine by refluxing with barium hydroxide\(^36\), the barium was removed by precipitation followed by filtration. The intermediate amino acid did not have to be isolated; addition of Neffken's\(^42\) reagent (N-carboethoxypthalimide) to the concentrated filtrate afforded the N-phthaloyl compound.

40
3-(2-tert. Butyl-4, 5-dimethoxyphenyl)-N-phthaloyl alanine (see also scheme 4) was converted with boron tribromide into the dihydroxy compound from which the phthaloyl group was removed by means of hydrazine\(^{43}\), a reaction in which the ortho-dihydroxy groups present were left undisturbed.

A survey of the synthesis of 6-tert. butyldopa is given in scheme 4 (chapter 3.6).

**EXPERIMENTAL PART**

4-tert. Butyl-5-methyl catechol.

A). By demethylation of the methyl ethers in the corresponding veratrorole.

4-tert. Butyl-5-methyl veratrorole was treated with BBr\(_3\) by the general method for demethylation (McOmie\(^{38}\)) at \(-60^\circ\). After stirring at this low temperature for 1.5 hr, the mixture was allowed to warm to room temperature. Next it was poured into ice and extracted with chloroform. Removal of the solvent, followed by addition of hexane, gave the catechol (m.p. 81-83\(^\circ\)). The structure was established by NMR spectroscopy (table 2).

The great difference with the m.p. (115-116\(^\circ\)) reported by Posp\(s\)i\(l\)\(^{19}\) induced us to reproduce his alkylation experiment.

B). By alkylation of 4-methyl catechol.

4-Methyl catechol was treated with tert. butyl alcohol in acetic acid as described by Posp\(s\)i\(l\)\(^{19}\) to give a mixture of alkyl-substituted catechols. TLC (1/4 mm of silica gel GF 254, elution with acetone-CCl\(_4\) (1:4)) showed that the mixture contained 3-tert. butyl-5-methyl catechol, 4-tert. butyl-5-methyl catechol and some 4-methyl catechol. Mixing with reference compounds (including the low-melting 4-tert. butyl-5-methyl catechol) gave no supplementary spots. On exposure of the chromatogram to the air overnight, the components showed different colours. The NMR spectrum of the reaction mixture showed a mixed spectrum of 3-tert. butyl-5-methyl catechol and 4-tert. butyl-5-methyl catechol in the ratio 2:3.

Recrystallization of the low-melting 4-tert. butyl-5-methyl catechol from hexane raised the melting point to 115-116\(^\circ\), a value fully consistent with that reported by Posp\(s\)i\(l\).

**Anal. calc.** for C\(_{11}\)H\(_{16}\)O\(_2\):

- C: 73.30% C, 8.8% H
- Founded: 73.3 % C, 8.8 % H.
N-Carboethoxyphthalimide (Nefkens reagent). This compound was synthesized according to Nefkens\(^2\) from phthalimide and ethyl chlorocarbonate. M.p. 89-90.5\(^0\) (lit. 82\(^0\), 87-89\(^0\)) after crystallization from benzene-petroleum ether; yield 60\%; NMR (CDCl\(_3\)): \(6\) 1.43 (3H, CH\(_3\)), 4.49 (2H, CH\(_2\)) and 7.65-8.15 (4H, aryl CH).

Anal. calc. for C\(_{11}\)H\(_9\)NO\(_4\) : 60.27% C, 4.14% H found : 60.7 % C, 4.3 % H.

3-(2-tert. Butyl-4, 5-dimethoxyphenyl)-N-phthaloylalanine. A mixture of 3 g of the corresponding N-benzoyl derivative (chapter 3.4) and aqueous Ba(OH)\(_2\) (Behringer\(^3\)) was refluxed under N\(_2\) for 5-5 days. The barium was precipitated by passing CO\(_2\) through the reaction mixture followed by acidification to pH 4 with dilute H\(_2\)SO\(_4\). After removal of the insoluble salts by filtration, the acidic solution was neutralized with aqueous bicarbonate and then evaporated to dryness under reduced pressure. The residual crude tert. butyl-dimethoxyphenylalanine was washed with ether (to remove the benzoic acid), suspended in 30 ml of water and adjusted to pH 6-7.

After addition of 0.7 g of sodium carbonate and 1.5 g of Nefkens reagent to the suspension, the flask was shaken for 15 min. Acidification of the filtered solution afforded the N-phthaloylaminobenzoyl acid, which was crystallized from ethanol-water. M.p. 191-198\(^0\) after prior changes of the crystalline structure at about 177\(^0\); yield ca. 50%; IR (CHCl\(_3\)): 1720 and 1780 cm\(^{-1}\) (C=O); NMR (CDCl\(_3\)): \(6\) 1.47 (9H, tert. butyl CH\(_3\)), 3.39 (3H, OMe), 3.83 (3H, OMe), 3.55-4.25 (mult., 2H, \(\beta\)-CH\(_2\)), 5.33 (center mult., 1H, \(\alpha\)-CH), 6.43 and 6.93 (singlets, 2H, aryl CH), 7.70 (center mult., 4H, phthaloyl CH), 10.0 (OH, COOH).

Anal. calc. for C\(_{22}\)H\(_{28}\)NO\(_6\) : 67.19% C, 6.12% H, 3.41% N found : 67.1 % C, 6.3 % H, 3.2 % N.

3-(2-tert. Butyl-4, 5-dihydroxyphenyl)-N-phthaloylalanine. The corresponding dimethoxy derivative (1 g) was treated with 8.75 g BBr\(_3\) in 25 ml methylene chloride by the above method for the cleavage of the methyl ethers in 4-tert. butyl-5-methylveratrole. The resultant mixture was poured onto ice-water and the methylene chloride removed in vacuo. The pale green N-phthaloyl-dihydroxyphenylalanine separated in the residual acidic aqueous layer. A chloroform-ethanol-petroleum ether (b.p. 80-100\(^0\)) mixture was used for recrystallizations, M.p. ca. 230\(^0\)(dec.); yield 75% IR (0.5% in KBr): 3480 cm\(^{-1}\) (aromatic OH), 1730 and 1780 (C=O); NMR (D\(_6\)-DMSO): \(6\) 1.33 (9H, tert. butyl CH\(_3\)), 3.55 (center mult., 2H, \(\beta\)-CH\(_2\)), 4.93 (center mult., 1H, \(\alpha\)-CH), 6.32 and 6.75 (singlets, 2H, aryl CH), 7.87 (center mult., 4H, phthaloyl CH).

Anal. calc. for C\(_{21}\)H\(_{21}\)NO\(_6\) : 65.78% C, 5.53% H, 3.65% N found : 65.4 % C, 5.5 % H, 3.7 % N.

3-(2-tert. Butyl-4, 5-dihydroxyphenyl)alanine, (6-tert. butyldopa). After 0.7 g of the above N-phthaloyl-dihydroxyphenylalanine had been refluxed with 0.25 g of hydrazine hydrate in 10 ml of ethanol under N\(_2\) for 1 hr (Sheehan\(^3\)), the ethanol was removed in vacuo and 9 ml of 2N HCl was added. The mixture obtained was heated at 50\(^0\) for 15 min and then cooled.

Next phthalazine was filtered off. The filtrate was neutralized with bicarbonate to pH 6 and the aqueous solution evaporated to a small volume, from which at pH 5.5 the 6-tert. butyldopa crystallized. The amino acid was purified by recrystallization from water with a trace of SO\(_2\) or from a solution, made by acidification of a suspension of the compound in water until all the solid had dissolved, followed by neutralization with bicarbonate to pH 5.5.

M.p. ca. 250\(^0\) (dec.); yield ca. 50% NMR (D\(_2\)O + DCI): 6 1.38 (9H, tert. butyl CH\(_3\)), 3.52 (center mult. 2H, \(\beta\)-CH\(_2\)), 4.46 (center mult. 1H, \(\alpha\)-CH), 6.89 and 7.09 (singlets, 2H, aryl CH).

Anal. calc. for C\(_{15}\)H\(_{19}\)NO\(_4\) : 61.65% C, 7.66% H, 5.53% N found after drying at 100\(^0\) (1 mm, PH\(_2\)O) for 24 hr : 61.5 % C, 7.6 % H, 5.6 % N.
3.6. SURVEY OF THE SYNTHESIS OF 6-TERT. BUTYLDOPA.

In scheme 4 the total synthesis of 6-tert. butyldopa is represented. Each number in parentheses indicate the chapter in which the reaction concerned is discussed and where the physical data on the reactants are given in the respective experimental part. The overall yield of 6-tert. butyldopa amounted to 4.5%.
LITERATURE

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CHAPTER 4

GEOMETRIC ISOMERISM OF $\alpha$-BENZAMIDOCINNAMATES
AND THEIR RELATED AZLACTONES.

4.1. INTRODUCTION

As early as 1900 Erlenmeyer and Plöchl synthesized unsaturated azlactones (4-arylidene-2-phenyl-2-oxazolin-5-ones) by the reaction of an aromatic aldehyde with hippuric acid under the influence of acetic anhydride with sodium acetate$^1)$. Usually, only one of the two possible geometric isomers of the azlactone can be isolated.

By another route Carter$^2)$ succeeded in 1941 in synthesizing both geometric isomers of 4-benzylidene-2-phenyl-2-oxazolin-5-one, using the two diastereoisomic N-benzoyl-3-methoxy-3-phenylalanines as starting materials. In 1952 Buckles and Filler$^3)$ followed a similar procedure to obtain the isomers. Tatsuoko$^4)$ (1950) found that an azlactone produced by the Erlenmeyer-Plöchl synthesis (the "stable azlactone") changes into the less stable isomer on treatment with concentrated hydrobromic acid at $0^\circ$C. Filler$^5,6)$ (1962) successfully applied this reaction to convert a number of stable azlactones into their geometric isomers unknown up till then.

In scheme 5 the work of the above authors is represented. The scheme also shows that the isomeric azlactones (Ia and Ib) can be converted into the corresponding $\alpha$-benzamidocinnamates (IIa and IIb) without changes in geometric configuration.
4.2. CIS-TRANS ASSIGNMENTS IN THE LITERATURE

A number of investigations have been carried out in the past to establish the geometric configuration of the molecules in either group of compounds shown in scheme 5; the results were conflicting.

The work by Buckles and Filler (1952) led them to assume that the group of stable compounds (scheme 5) would possess the cis configuration.

*) The cis and trans nomenclature used to describe these compounds is based -as in cinnamic acid- on the mutual arrangement of the phenyl and carbonyl group.

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This assumption was based on U.V. spectral data and a comparison of the melting points of the \( \alpha \)-benzamidocinnamic acids (IIIa and IIIb) with those of model compounds (4-benzamido-2-biphenylicarboxylic acid and 2-benzamido-4-biphenylicarboxylic acid).

On the basis of elimination reactions, Kochetkov\(^7\) concluded that the structures of (Ia) and (Ib) are opposite to those proposed by Buckles et al.. The erythro and threo isomers of N-benzoyl-3-phenylserine were treated with acetic anhydride to give the two geometric isomers of benzylidene-oxazolinone (Ia and Ib).

According to that author the configuration of the azlactones is determined by trans-elimination of a molecule of water from the intermediate saturated hydroxyazlactone. Under the reaction conditions of the elimination partial conversion of the cis into the trans compound was observed. Kochetkov\(^7\) found support for this assignment of structure in the acid strengths of the derived unsaturated hydroxamic acids. Filler\(^8\) observed that each of the diastereoisomeric methyl ethers of N-benzoyl-3-phenylserine gave mixtures of the azlactones in acetic anhydride. In his opinion it is doubtful that configurational integrity is maintained in the reactions
performed by Kochetkov in the same medium.

Recently Brocklehurst\(^9\) made an attempt to establish the structures of the two geometric isomers (Ia) and (Ib) by using NMR data of the corresponding \(\alpha\)-benzamidocinnamates. The chemical shifts of the olefinic protons \((H_\beta\) in (IIa) and (IIb) were determined approximately from integration ratios; the values obtained were compared with the chemical shift of \(H_\beta\) in cis- and trans-methyl cinnamate.

**Scheme 6** NMR data from Brocklehurst.

<table>
<thead>
<tr>
<th>cis compounds</th>
<th>trans compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{MeOOC} - H)</td>
<td>(\text{MeOOC} - \text{NHCOPh})</td>
</tr>
<tr>
<td>(H_\beta)</td>
<td>(H_\beta)</td>
</tr>
<tr>
<td>((5691))</td>
<td>((5728-765))</td>
</tr>
<tr>
<td>((5771))</td>
<td>((5771-800))</td>
</tr>
</tbody>
</table>

Brocklehurst pointed out that the influence of the benzamido group on the chemical shift of \(H_\beta\) in the \(\alpha\)-benzamidocinnamates would be limited, and that the relative positions of the \(H_\beta\) signals would be mainly controlled by the deshielding effect of the cis or trans ester group. Therefore, he assigned the geometric structures as indicated in scheme 6. (see also chapter 4.4.).

Galantay's observation\(^10\) of only an extremely small change in chemical shift of protons in \(\gamma\)-position on introduction of an \(\alpha\)-benzamido group supported Brocklehurst's assumption that the effect of a benzamido substituent would be limited indeed.

\[ \text{Galantay} \]

\[ (51.84) \quad (52.12) \]
\[ (51.85) \quad (52.15) \]

It should be noted that Galantay has not provided conclusive evidence that the assignment of the signals \((51.85\) and 2.15) to the methyl groups is not opposite to that given in the latter figure.
4.3. SYNTHESIS AND NMR DATA OF THE GEOMETRIC ISOMERS OF α-BENZAMIDO-3, 4-DIMETHOXYCINNAMATE AND THEIR RELATED AZLACTONES.

In the preparation of 6-alkyl derivatives of dopa four azlactones carrying an alkyl substituent in the arylidene group (chapter 2.2 and 3.4) were obtained via the Erlenmeyer-Plöchl synthesis. Although in the case of the 6-tert.butyl compound two different routes were followed, the azlactones isolated were fully identical (chapter 3.4). In order to gain insight into the geometric structures of these azlactones, the problem of cis-trans isomerism was first studied in the geometric isomers of α-benzamido-3, 4-dimethoxycinnamate and in the corresponding azlactones (scheme 7). The synthesis of the four compounds was carried out by the methods indicated in scheme 5 for the unsubstituted derivatives. The stable isomer (IVa), obtained by Erlenmeyer-Plöchl synthesis, was converted with concentrated hydrobromic acid into the metastable geometric isomer (IVb) as described for analogous compounds\(^4,5\). Hydrolysis of (IVa), followed by esterification, yielded the corresponding ethyl α-benzamidocinnamate (Va), termed the stable isomer. Basic alcoholysis of (IVb) yielded the benzamidocinnamate (Vb), called metastable isomer because of its preparation from the metastable azlactone.

**SCHEME 7** Dimethoxy azlactones and esters

\[
\begin{align*}
\text{AZLACTONES} & \\
\text{IVa stable isomer (m.p.1495-151°)} & \quad \text{IV-trans} \\
\text{IVb metastable isomer (m.p.147.5-1485°)} & \quad \text{IV-cis} \\
\text{α-BENZAMIDO-} & \\
\text{CINNAMATES} & \\
\text{Va stable isomer (m.p.118-119.5°)} & \quad \text{V-trans} \\
\text{Vb metastable isomer (m.p.141-142°)} & \quad \text{V-cis}
\end{align*}
\]

NMR spectral data are shown in table 3; the region of the aromatic protons in the spectra is represented in figure 1. The splitting pattern (ABC system) described by Sasaki\(^11\) of the 2-, 5-, and 6-protons in the dimethoxyphenyl
FIGURE 1  NMR spectra* of azlactones (IV_a,b) and esters (Va,b)

*) Solvent CDCl₃; standard TMS (δ=0); temp ca 38°
group partly coincided with the splitting pattern of the aromatic protons in the other phenyl ring present in the molecule. The latter pattern was found in about the same region in the NMR spectra of all azlactones and esters given in tables 3 and 4. The 2'- and 6'-protons gave a multiplet between δ 7.7 and 8.3 (see fig. 1, spectrum of IVb), whereas the multiplet of the 3'-, 4'- and 5'-protons appeared between δ 7.3 and 7.7 (fig. 1, spectrum of Vb).

For the compounds in schema 7 the chemical shift of the singlet of the olefinic proton, often partly hidden by the splitting patterns of the aromatic protons, was unambiguously established by running the NMR spectra of the corresponding azlactones and esters in which the olefinic proton had been replaced by deuterium. The signals in figure 1 indicated by -CH= were absent in the spectra of the β-deuterated azlactones and esters. The preparation of the compounds deuterated in β-position was performed in accordance with the procedures described above, starting from α-deuterioveratraldehyde which was obtained by a method, recently reported by Axenrod\textsuperscript{12)} for the synthesis of α-D-benzaldehydes.

\[
\begin{align*}
\text{MeO} & \quad \text{MeO} & \quad \text{MeO} & \quad \text{MeO} \\
\text{COOH} & \quad \text{Cl} & \quad \text{CONHC}(\text{CH}_3)_2 & \quad \text{CONHC}(\text{CH}_3)_2 \\
\text{SOCl}_2 & \quad \text{MeO} & \quad \text{MeO} & \quad \text{MeO} \\
\text{(CH}_3)_2\text{CNH}_2 & \quad \text{(CH}_3)_2\text{CNH}_2 & \quad \text{(CH}_3)_2\text{CNH}_2 & \quad \text{(CH}_3)_2\text{CNH}_2 \\
\text{(β-D)IVb} & \quad \text{(β-D)IVa} & \quad \text{(β-D)IVa} & \quad \text{(β-D)IVa} \\
\text{(β-D)Vb} & \quad \text{(β-D)Vb} & \quad \text{(β-D)Vb} & \quad \text{(β-D)Vb}
\end{align*}
\]

A comparison of the U.V. spectra of (IVa, b) in ether and (Va, b) in ethanol reveals differences in the intensity of absorption between the geometric isomers (see experimental part). It is not impossible that the strongly decreased intensity, observed by Buckles and Filler\textsuperscript{3)} for the unsubstituted less stable azlactone (Ib) was due to a partial conversion of (Ib) into the ester by reaction with the solvent (ethanol).
TABLE 3  NMR data of the geometric isomers of \( \alpha \)-benzamido-3,4-dimethoxy-cinnamate and their related azlactones.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shifts (^a) (( \delta ), p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{CH}_3\text{CH}_2 )</td>
</tr>
<tr>
<td>IVa</td>
<td>-</td>
</tr>
<tr>
<td>IVb</td>
<td>-</td>
</tr>
<tr>
<td>Va</td>
<td>1.33; 4.30</td>
</tr>
<tr>
<td>Vb</td>
<td>1.13; 4.21</td>
</tr>
</tbody>
</table>

\(^a\) Solvent CDCl\(_3\); standard TMS; temperature ca. 38\(^\circ\) C. \(^b\) \( J_{26} = 2 \text{ c.} /\text{sec.}, J_{56} = 8-9 \text{ and } J_{28} = 0 \text{ c.} /\text{sec.} \)
\(^c\) \( 5, 6, 9 \) is the centre of an ABC system in the 6.85-6.95 region.

EXPERIMENTAL PART

Stable 4-(3,4-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-one (IVa). Prepared by the Erlenmeyer-Plochl synthesis\(^{13}\); m.p. 149.5-151\(^\circ\) (lit.\(^{13}\) 149-150\(^\circ\)); yield 70%.

Metastable 4-(3,4-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-one (IVb). After suspending 1 g of (IVa) in 20 ml of 48% aqueous HBr, gaseous HBr was passed through the solution at 0\(^\circ\) for 1.5 hr.\(^{4,5}\) The red suspension was allowed to stand overnight at 0\(^\circ\). The precipitate was filtered, and then filtered. The orange precipitate was purified by crystallization from ethanol or benzene. M.p. 147.5-148.5\(^\circ\); yield ca. 50%.

Anal. calc. for C\(_{18}\)H\(_{16}\)NO\(_3\): 69.89\% C, 4.89\% H, 4.53\% N
found: 69.7\% C, 4.9\% H, 4.6\% N.

Stable ethyl \( \alpha \)-benzamido-3,4-dimethoxycinnamate (Va). Heating 0.01 mole of (IVa) with 12 ml 5% aqueous NaOH and 12 ml of ethanol, followed by acidification, gave the benzamidocinnamic acid.

A mixture of 0.01 mole of the acid, 10 ml of ethanol and 3 drops of concentrated H\(_2\)SO\(_4\) was refluxed for 15 hr, diluted with water and neutralized with aqueous bicarbonate. The resulting solution was left standing for a while, the ester crystallized. (Va) was purified by recrystallization from toluene-petroleum ether. M.p. 118-119.5\(^\circ\) (lit.\(^{14}\) 118-119\(^\circ\)); yield ca. 50%.

Metastable ethyl \( \alpha \)-benzamido-3,4-dimethoxycinnamate (Vb). A mixture of 0.001 mole of (IVb), 5 ml of 4% aqueous NaOH and 5 ml of ethanol was stirred at 0\(^\circ\) for 1 hr. The solution obtained was diluted with water and then neutralized to pH 7.5 with dilute HCl. The precipitate was filtered off and crystallized from toluene-petroleum ether (b.p. 80-80\(^\circ\)).

M.p. 141-142\(^\circ\); yield ca. 60%.

Anal. calc. for C\(_{20}\)H\(_{21}\)NO\(_3\): 67.59\% C, 5.99\% H, 3.84\% N
found: 67.4\% C, 6.1\% H, 4.3\% N.
UV spectra $\lambda_{\text{max}}$ m$\mu$ (log $e_{\text{max}}$)
In imitation of Baisi,$^{15}$ only the principal maxima of the azlactones are recorded. The spectra of (IVA, b) were run in ether and those of (Va, b) in ethanol.

(IVA): 264 (4.24), 399 (4.59); (IVb): 264 (4.03), 406 (4.59); (Va): 295 (sh) (4.15), 324 (4.28);
(Vb): 320 (4.36).

N-tert. Butylveratramide (N-tert. butyl-3, 4-dimethoxybenzamide). Veratric acid, prepared from veratraldehyde according to Raiford,$^{16}$ was converted with SOCl$_2$ into the acid chloride as described by Gutzke$^{17}$ (m. p. 67-71$^0$; yield 85%). The acid chloride was treated with tert. butylamine$^{18}$ by the method of Axenrod.$^{12}$ The white precipitate, which contained the sparingly ether-soluble amide, was filtered off and suspended in water. The amide was isolated by extraction with large amounts of ether and evaporation of the etheral layer. The crude product thus obtained was crystallized from petroleum ether.

M. p. 126-128$^0$; yield 74%; IR (CHCl$_3$): 3460 cm$^{-1}$ (NH), 1660 (C=O)

$\alpha$-Deuterioveratraldehyde. The preparation was analogous to the synthesis by Axenrod$^{12}$ of $\alpha$-deuteroanisaldehyde. Reduction of 0.02 mole of amide with LiAlD$_4$ in 240 ml of ether and 50 ml of THF afforded the crude aldime, which was immediately subjected to a further treatment with dilute HCl.

The final reaction product appeared to be a mixture of the starting amide and the deuterated veratraldehyde. By distillation a fairly pure aldehyde could be isolated.
B. p. 150-160$^0$/ca. 12 mm; yield 45%; IR (CHCl$_3$): 2070 and 2120 cm$^{-1}$ (C-D stretching doublet$^{15}$), 1670 (C=O).

$\beta$-Deuterated azlactones and $\alpha$-benzamidocinnamates. $\alpha$-Deuterioveratraldehyde was converted by the Erlenmeyer-Plochh synthesis into $\beta$-D-4-(3, 4-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-one [($\beta$-D) IVA], from which the deuterated metastable azlactone [($\beta$-D) IVb] and subsequently the two deuterated esters [($\beta$-D) Va] and [($\beta$-D) Vb] were prepared as described in this experimental section.

4.4. DISCUSSION OF THE NMR DATA AND CIS-TRANS ASSIGNMENT.

The primary cause of the difference between the chemical shifts of the olefinic protons in the benzamidocinnamates (Va) and (Vb) has to be found in the different shielding effects of the ester and benzamido substituents in cis and in trans position on $H_5$. Assuming simple additivity of substituent effects on the chemical shift of olefinic protons, Matter and Pascual$^{19, 20}$ derived shielding parameters for several functional groups. Although deviations$^{19}$ from the additivity principle were observed, the chemical shifts calculated by means of the substituent shielding increments (in this thesis $\Delta$) were in many cases in good agreement with the experimental values.

$$5H = 5H_{\text{ethylene}} + \Delta(R_1)_{\text{cis}} + \Delta(R_3)_{\text{trans}} + \Delta(R_3)_{\text{cis}}$$
For the calculation of the chemical shift of $H_B$ in (Va) and (Vb) it was necessary to determine the magnitude of the effect of a cis or a trans benzamido substituent on the chemical shift of an olefinic proton ($R_1$ or $R_2 = -\text{NHCOPh}$). On this subject reports are scarce; the work of Galantay\textsuperscript{10} (chapter 4.2) provides little information.

Zanger\textsuperscript{21} investigated the influence of an acetamido substituent on the chemical shift of an ortho proton in benzene derivatives. In a number of ortho-substituted N-acylanilines a considerably increased deshielding of the proton ortho to the acylamino group appeared to occur, as is shown in the following example:

\[
\begin{array}{c}
\text{calc. for } \delta H_2 \text{ : } 720 \\
\text{found : } 832
\end{array}
\]

According to Zanger\textsuperscript{21} one of the causes of the strong deshielding effect is the steric influence of the ortho substituent, the molecule preferring that conformation in which the ortho proton lies in the deshielding zone of the carbonyl group.

Zanger’s work raised the presumption that in compounds (Va) and (Vb) the steric influence of the ester group would enable the benzamido group to deshield $H_B$ considerably.

It was therefore necessary that the $\alpha$-benzamido $\beta$-unsaturated carbonyl moiety was present in the model compounds (VI and VIII) for the estimation of the benzamido substituent effect.

The chemical shifts of the $\beta$-protons in the methyl ester of $\alpha$-benzamidoacrylic acid (VI, for its synthesis see experimental part) were compared with those in methyl acrylate (VII).

\[
\begin{bmatrix}
\delta 6.01 \\
\delta 6.80
\end{bmatrix}
\]

\[
\begin{bmatrix}
\delta 5.82 \\
\delta 6.38
\end{bmatrix}
\]

Application of the additivity principle after the assignment to $H_A$ and $H_B$ of the chemical shifts found for the olefinic protons in (VI), provided two possibilities for the magnitude of the benzamido substituent effect in the cis $[\Delta(b)_{\text{cis}}]$ and in the trans position $[\Delta(b)_{\text{trans}}]$ with respect to $H_B$:

\textsuperscript{1)} Varian spectra catalogue; spectrum No. 64.
(A) chem. shift $H_A = \delta 6.01$ and $H_B = \delta 6.80$

\[
\begin{align*}
\delta H_A &= 6.01 = 6.38 + \Delta (b)_{\text{trans}} \quad \text{i.e. } \Delta (b)_{\text{trans}} = -0.37 \\
\delta H_B &= 6.80 = 5.82 + \Delta (b)_{\text{cis}} \quad \text{i.e. } \Delta (b)_{\text{cis}} = +0.98
\end{align*}
\]

(B) chem. shift $H_A = \delta 6.80$ and $H_B = \delta 6.01$

\[
\begin{align*}
\delta H_A &= 6.80 = 6.38 + \Delta (b)_{\text{trans}} \quad \text{i.e. } \Delta (b)_{\text{trans}} = +0.42 \\
\delta H_B &= 6.01 = 5.82 + \Delta (b)_{\text{cis}} \quad \text{i.e. } \Delta (b)_{\text{cis}} = +0.19
\end{align*}
\]

In order to make a choice between (A) and (B) the chemical shift of the olefinic proton in 3-benzamidocoumarin (VIII, for its synthesis see experimental part) was compared with that of the corresponding proton at the 4-position in coumarin (IX).

![VIII](5883) ![IX](5780)

Introduction of a benzamido substituent in cis position shifted the signal of the olefinic proton from $\delta 7.80$ to $\delta 8.83$, which means $\Delta (b)_{\text{cis}} = +1.03$. Introduction of a 3-acetamido group \(^{[23]}\) also resulted in a considerable downfield shift of the 4-proton ($\delta 8.68$).

The value of $\Delta (b)_{\text{cis}} = +1.03$, determined from the coumarins, agreed well with the benzamido substituent effect in cis position ($+0.98$) calculated above [ assumption (A) ], so that preference was given to the $\Delta s$ of (A) over those of (B).

The electron-attracting character of the benzamido substituent led Brocklehurst \(^{[9]}\) to believe that the combination of a highly positive $\Delta_{\text{cis}}$ and a negative $\Delta_{\text{trans}}$ is not likely to occur. It should, however, be borne in mind that the range of applications of "unlikely" $\Delta s$ is restricted to $\alpha$-benzamidoacrylic acid derivatives; besides, for comparison of the calculated chemical shifts with the experimental values, the latter should be measured in deuterochloroform. The deviating \(^{[19]}\) magnitude of the benzamido substituent effect in cis and trans position can be accounted for by the fact that the change of the substituent effect of the ester group on the $H_B$ signal as a result of the interaction with the introduced benzamido group, is included in the calculated values of these $\Delta s$. 

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In addition, it was to be expected that the $\delta(b)$s calculated from (VI) would deviate from possible $\delta(b)$s derived from ethylene derivatives with the benzamido substituent in a more isolated position. A similar discrepancy, to be attributed to an interaction of substituents at the $\alpha$-carbon atom in an acrylic acid derivative, was observed on introduction of a second $\alpha$-ester group into ethyl trans-3,4-dimethoxycinnamate (X).

![Chemical structures](image)

The experimental chemical shift of $H_B$ (measured in 10% solution in CDCl$_3$) in trans-3,4-dimethoxycinnamate (X) was in good agreement with the shift calculated with the aid of Matter's substituent shielding parameters.$^{19}$ Introduction of a second $\alpha$-ester group (XI) caused only a minor alteration in the chemical shift, while the statistical average of the substituent effect of a trans-COOR group on an olefinic proton (+0.46)$^{19,20}$ would suggest a downfield shift. On comparing the experimental chemical shifts of $H_B$ it was seen that $\Delta(COOR)_{\text{trans}} \approx +0.01$.

Thus, application of the additivity principle to the experimental chemical shifts, provided a $\Delta_{\text{trans}}$ that was 0.46 smaller than the corresponding "isolated" substituent effect on $H_B$.

Choice of (A) defines the position of the $H_B$ signals in the NMR spectrum of (VI): chemical shift $H_A = \delta 6.01$ and $H_B = \delta 6.80$. Replacement of $H_A$ or $H_B$ in this benzamidoacrylate with a 3,4-dimethoxyphenyl group allowed the calculation of the chemical shift of $H_B$ in the $\alpha$-benzamidocinnamates (V-cis and V-trans, scheme 7) with the aid of Matter's$^{19}$ substituent effect of an aryl group on an olefinic proton in $\alpha$-position (+1.38).

\[
\text{calc. } \delta H_B \text{ in (V-trans)} = \delta H_A + \Delta(\text{aryl})_{\alpha-*} = 6.01 + 1.38 = 7.39
\]
\[
\text{calc. } \delta H_B \text{ in (V-cis)} = \delta H_B + \Delta(\text{aryl})_{\alpha-*} = 6.80 + 1.38 = 8.18
\]

There was a reasonable agreement between the calculated values and the observed values ($\delta 7.44$ and $8.00$, table 3), provided that the trans configuration was assigned to the stable isomer (Va) and the cis structure to the less stable isomer (Vb).
Proceeding from the chemical shifts found by Brocklehurst for $H_B$ in cis- and trans-methyl cinnamate, it was possible to calculate the shift of $H_B$ in the corresponding $\alpha$-benzamido derivatives (see scheme 6) using the benzamido substituent effects in cis and trans position [assumption (A): + 0.98 and - 0.37, respectively].

\[
\begin{align*}
\text{calc. } \delta H_B \text{ in cis-} \alpha\text{-benzamidocinnamate} &= 6.91 + \Delta(b)_{\text{cis}} = 7.89 \\
\text{calc. } \delta H_B \text{ in trans-} \alpha\text{-benzamidocinnamate} &= 7.71 + \Delta(b)_{\text{trans}} = 7.34
\end{align*}
\]

The calculated values were within the range experimentally established by Brocklehurst for the $H_B$ signal (see scheme 6). The cis-trans assignment for the benzamidocinnamates proposed by that author should, however, be inverted. With regard to both the $\alpha$-benzamido-3, 4-dimethoxycinnamates (scheme 7, Va, b) and the $\alpha$-benzamidocinnamates (scheme 6) the above reasoning leads to the conclusion that in the stable esters the (dimethoxy)phenyl group is in trans position to the ester group, i.e. (Va) = (V-trans). In the metastable esters with the $H_B$ signal at the lowest field the (dimethoxy)phenyl group is cis to the ester group, i.e. (Vb) = (V-cis).

The cis-trans assignment was confirmed by the large upfield shift of the protons of one of the methoxy groups in the NMR spectrum of (Va) (see table 3). A comparison of the methoxy protons in the compounds (X) and (XI) showed that it is not probable that the upfield shift would be due to the influence of an ester group cis to the aryl ring. For steric reasons the dimethoxyphenyl group in either $\alpha$-benzamidocinnamate is not expected to be fully coplanar with the double bond (Sandris, Frasca). On consideration of the influence of a cis benzamido group on the protons of the methoxy groups in the aryl ring two types of only approximately coplanar conformations are to be distinguished.

When $R=H$ there seems to be no reasons to assume that the molecule has preference for one of the depicted conformations. Molecular models showed that in conformations of type P the protons of particularly the 3-methoxy group may be situated in the shielding zone of the benzoyl group. The assumption that
introduction of an alkyl group into the 6-position in (Va) would give rise to preference to conformations (R=alkyl) of type P over those of type Q because of steric hindrance in the latter, proved consistent with the experimentally established increase in the upfield shift of the signal from the protons of one of the methoxy groups in the nuclear-alkyl-substituted benzamidocinnamates (chapter 4.5).

Assignment of geometric structure to the benzamidocinnamates implies (see scheme 5) that also the geometric configuration of the azlactones (IVa) and (IVb) is defined. The stable azlactone obtained in the Erlenmeyer-Plöchl synthesis has the trans configuration—the dimethoxyphenyl group is trans to the oxazolinone carbonyl—, the cis configuration being assigned to the less stable isomer (IVb). From the NMR spectra (table 3) it appeared that under the deshielding influence of the cis cyclic carbon-nitrogen double bond the $H^B$ signal shifts further downfield than when the oxazolinone carbonyl group is cis to $H^B$.

The large downfield shifts of $H^2$ and $H^6$, apparently due to the situation of these protons in the deshielding region of the oxazolinone ring, indicate a nearly planar structure of the azlactones. The great difference in chemical shift between $H^2$ and $H^6$ can only be ascribed to an unaccountable influence of the proximity of the methoxy oxygen atom ortho to $H^2$. In the NMR spectrum of the 3,4-methylenedioxy analogue of (IVa) the same considerable difference in chemical shift was observed ($\delta H^2 : 8.12, \delta H^6 : 7.48$). Restriction of rotation of the aryl ring in the azlactones, resulting in a preference for conformation P in which $H^2$ is the more deshielded proton, is not likely to occur; measurements of the NMR spectra of (IVa) and (IVb) at about 140° in dimethyl diethylene glycol ether also revealed a large difference between the chemical shifts of $H^2$ and $H^6$.

6-Alkyl derivatives of the azlactone (IVa), however, could be expected to exist preferentially in conformations of type P. This was confirmed experimentally by an increase of the downfield shift of the $H^2$ signal in the NMR spectra of the alkyl-substituted compounds. (see chapter 4.5)
EXPERIMENTAL PART

Methyl α-benzamidoacrylate (VI). α-Benzamidoacrylic acid was prepared from benzamide and pyruvic acid in trichloroethylene according to Wieland. M. p. 156-158°; NMR (D₈-acetone): δ 6.08 (doublet) and 6.80 (singlet) (2H, olefinic CH), 7.5-8.2 (aryl CH), 8.7-9.4 (COOH and NH), on addition of D₂O a singlet appeared at δ 6.08. By addition of NaOH 50 mg of the α-benzamidoacrylic acid could be dissolved in water. Next the solution was adjusted to pH 6 by means of 2N HNO₃. Addition of 1.5 ml of 4% aqueous AgNO₃, followed by shaking, caused a white silver salt to precipitate. The solid was filtered off and, for some min, dried in vacuo (50 mg; m. p. 178° with decomposition). The fresh silver salt was refluxed with an excess of methyl iodide in anhydrous ether for 2 hr. The produced AgI was removed by filtration, and the solvent of the filtrate evaporated. The crude ester thus obtained was not further purified because the compound was expected to have a low stability. NMR (CDCl₃): δ 3.88 (CH₃), 6.01 (doublet) and 6.80 (singlet) (2H, olefinic CH), 7.4-8.0 (aryl CH and NH), the splitting of the δ 6.01 proton is probably due to coupling with NH (J = 1-2 c./sec.).

3-Benzamidocoumarin (VIII). Salicylaldehyde was heated with hippuric acid in acetic anhydride under the influence of sodium acetate (Lambooy) to give a mixture of the corresponding azlactone and some benzamidocoumarin. Treatment of the mixture with 10% aqueous NaOH according to Erlenmeyer, followed by acidification and filtration of the precipitate which was washed with aqueous sodium carbonate, gave 3-benzamidocoumarin in low yield. On crystallization from ethanol the m. p. was found to be 175.5-176.5° (lit. 176°).

Ethyl trans-3,4-dimethoxycinnamate (X). Prepared by esterification of the corresponding acid; m. p. 56-57.5° (lit. 55.5°).

Diethyl 3,4-dimethoxybenzylidenemalonate (XI). This compound was synthesized (as described for the monomethoxy derivative) from veratrinaldehyde and diethyl malonate in benzene with some piperidine. Distillation of the reaction mixture yielded an oil which solidified on standing. Crystallization from ethanol gave (XI); m. p. 48-49.5°.
Anal. calc. for C₁₁H₁₂O₆: 62.33% C, 6.54% H
found : 62.5 % C, 6.4 % H.

4.5. GEOMETRIC CONFIGURATION OF THE STABLE ALKYL-SUBSTITUTED AZLACTONES.

The synthesis of the azlactones alkyl-substituted in the benzylidene group (alkyl = methyl, ethyl, isopropyl or tert. butyl) was discussed in chapters 2 and 3, while the corresponding stable ethyl α-benzamidocinnamates were prepared from the azlactones as described previously. Table 4 lists the NMR spectral data.
TABLE 4  NMR data of the stable 2-alkyl-α-benzamido-4,5-dimethoxychinna-
mates and their related azlactones.

\[
\begin{align*}
\text{MeO} & \quad \text{CH}_2 \text{CH}\beta \quad \text{N} \quad \text{Ph} \\
(\text{IV-R})^a & \quad \text{MeO} & \quad \text{CH}_2 \text{CH}\beta \quad \text{N} \quad \text{HCOCH}_2\text{CH}_3 \\
(\text{V-R})^a & \quad \text{MeO} & \quad \text{CH}_2 \text{CH}\beta \quad \text{N} \quad \text{HCOPh}
\end{align*}
\]

( Me=methyl, Et=ethyl, iPr=isopropyl and tBu=tert. butyl )

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shifts ( \delta ) (p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R )</td>
</tr>
<tr>
<td>IV-Me</td>
<td>2.31</td>
</tr>
<tr>
<td>IV-Et</td>
<td>1.27;2.84</td>
</tr>
<tr>
<td>IV-iPr</td>
<td>1.29;3.50</td>
</tr>
<tr>
<td>IV-tBu</td>
<td>1.53</td>
</tr>
<tr>
<td>Va(^c)</td>
<td>-</td>
</tr>
<tr>
<td>V-Me</td>
<td>2.33</td>
</tr>
<tr>
<td>V-Et</td>
<td>1.23;2.71</td>
</tr>
<tr>
<td>V-iPr</td>
<td>1.26;3.22</td>
</tr>
<tr>
<td>V-tBu</td>
<td>1.43</td>
</tr>
</tbody>
</table>

\(^{a}\) In numbering the protons in the alkyl-dimethoxyphenyl group the official nomenclature was not followed to avoid confusion in comparing with the corresponding protons mentioned in table 3. \(^{b}\) Solvent CDCl\(_3\); standard TMS; temperature ca. 38\(^\circ\). \(^{c}\) See table 3.

The \( \text{H}_8 \) signal as well as the \( \text{H}_{(5)} \) and \( \text{H}_{(2)} \) signals appeared in the NMR spectrum as singlets; assignments of the chemical shifts were made by a comparison with the NMR data in table 3.

The downfield shift of the \( \text{H}_{(2)} \) signal in the azlactones in relation to the \( \text{H}_2 \) signal in the non-alkyl-substituted azlactones may be explained by the assumption that the alkyl-substituted compounds prefer conformations of type P to that of type Q (see chapter 4.4) because in the latter strong steric hindrance would occur. In conformation P the oxazolinone moiety is expected to deshield \( \text{H}_{(2)} \) to a higher extent than when the molecule exists in conformation Q.

The chemical shifts of \( \text{H}_{(5)} \) in the azlactones and esters, given in table 4, agreed well with the corresponding shifts of \( \text{H}_5 \) in table 3 when the substituent effect of an ortho-alkyl group according to Ballantine\(^{32}\) was taken into consideration.
Ortho substitution in an aromatic ring will in general cause an olefinic proton in α-position to shift downfield\(^{19}\). This implied the appearance of the \(H_8\) signal in table 4 further downfield than the corresponding signal in the non-alkyl-substituted compounds in table 3. On the basis of this condition the alkyl-substituted azlactones (IV-alkyl) and esters (V-alkyl) could only be correlated with the trans-azlactone (IVa) and the trans-ester (Va), respectively. In the attempt to relate the chemical shift of \(H_8\) in table 4 to that of one of the geometric isomers stated in table 3, it was taken into account that the azlactones (IV-alkyl) retain their configurations on conversion into the corresponding esters (V-alkyl).

Both series of table 4 demonstrate a downfield shift of \(H_8\) when the alkyl substituent increases in bulk, the largest difference being seen when the alkyl changes from isopropyl to tert. butyl. An analogous course of the chemical shift was found in a series of 2-aryl-1,3-indandiones (Bruynes\(^ {33}\)), in the 6-alkylveratraldehydes reported in this thesis and on comparison of the NMR data of some alkyl-substituted catechols.

\[ \text{Me(OH)}_2 \text{Me(OH)}_2 \text{alkyl} \]

In 6-Me-, 6-Et-, 6-iPr- and 6-tBu-veratraldehyde the chemical shift of the aldehydic proton amounted to \(\delta\) 10.12, 10.21, 10.18 and 10.51, respectively.

A comparison of the chemical shift of the protons of the 5-methyl group in 4-isopropyl-5-methylcatechol (\(\delta\) 2.12) with that in the corresponding 4-tert. butyl derivative (\(\delta\) 2.38, see table 2) showed a downfield shift of the methyl protons identical to the shift of \(H_8\) in (V-alkyl) caused by the change from an isopropyl to a tert. butyl group.

The trans configuration proposed for the alkyl-substituted esters and hence also for the corresponding azlactones was confirmed by the good agreement between the chemical shift of the ethyl protons of the ester group in (V-alkyl) and that of the trans-ester (Va). The considerable difference in chemical shift between the protons of the two methoxy groups was already attributed in chapter 4.4 to the presence of a benzamido group cis to the aryl-ring, supporting the assignment of the trans configuration as well.

Also the UV spectra (see experimental part) of the alkyl-substituted compounds bore the greatest resemblance to those of the non-alkyl-substituted trans isomers.

The diminishing intensity of the absorption of the maximum at 400-408 m\(\mu\) (according to Basi\(^{18}\)) the chromophore : Ar-CH=C=N=C-Ph afforded an indication that the deviation from a planar structure of the azlactones increases as the alkyl substituent becomes bulkier.

As to the structure of the esters a similar deviation was indicated by the decrease in intensity and the distinct shift of the absorption maximum at 320-330 m\(\mu\) to a shorter wavelength.
The above elucidation of the structures of a number of azlactones carrying different substituents may suggest that, in the majority of cases, the trans configuration should be assigned to azlactones obtained by usual Erlenmeyer–Plöchl procedure from substituted benzaldehydes and hippuric acid.

**EXPERIMENTAL PART**

4-(2-Alkyl-4, 5-dimethoxybenzylidene)-2-phenyl-2-oxazolin-5-ones (IV-alkyl)
See experimental parts of chapter 2.2 and 3.4.

**Ethyl 2-alkyl-α-benzamido-4, 5-dimethoxycinnamates (V-alkyl).** For alkyl=methyl and tert. bytyl, see chapter 3.4, experimental part. The esters with alkyl=ethyl and isopropyl were synthesized from the corresponding azlactones, as described for compound (Va) in the experimental part of chapter 4.3.

**Ethyl α-benzamido-2-ethyl-4, 5-dimethoxycinnamate (V-Et);**
m.p. 120.5-121.5°C.
Anal. calc. for C₂₂H₂₆NO₅ : 68.91% C, 6.57% H, 3.45% N.
found : 68.8 % C, 6.6 % H, 3.4 % N.

**Ethyl α-benzamido-2-isopropyl-4, 5-dimethoxycinnamate (V-IP);**
m.p. 167-168°C.
Anal. calc. for C₂₃H₂₇NO₅ : 69.49% C, 6.85% H, 3.52% N.
found : 69.4 % C, 6.7 % H, 3.6 % N.

**UV spectra** \( \lambda_{\text{max}} \) m\( \mu \) (log \( \varepsilon_{\text{max}} \))

In imitation of Bassi\(^\text{15}\)) only the principal maxima of the azlactones are mentioned. The UV spectra of the azlactones were run in ether and those of the esters in alcohol.

(IV-Me) : 267(4.17), 408(4.55); (IV-Et) : 267(4.16), 407(4.54); (IV-iP) : 267(4.15), 407(4.53); (IV-tB) : 267(4.11), 406(4.44).
(V-Me) : 295(sh)(4.06), 332(4.15); (V-Et) : 295(sh)(4.08), 331(4.15); (V-iP) : 294(sh)(4.03), 327(4.09); (V-tB) : 294(sh)(4.04), 320(4.02).
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CHAPTER 5

INHIBITION OF DOPA DECARBOXYLASE IN VITRO
BY 6-ALKYL DERIVATIVES OF DOPA

5.1. INTRODUCTION

The important role the enzyme dopa decarboxylase plays in the biosynthesis of noradrenaline and adrenaline has already been mentioned in chapter 1. Dopa decarboxylase is widely spread in mammalian tissues (Sandler\(^1\), Patel and Burger\(^2\)); extracts of guinea pig kidney and rat liver are frequently used for studies in vitro.

In view of the wide range of specificity of the enzyme Lovenberg\(^3\) proposed to change the name dopa decarboxylase into "aromatic L-amino acid decarboxylase", a term which has, however, not gained general acceptance\(^4\).

The cofactor for the activation of dopa decarboxylase is pyridoxal phosphate, which is associated with the enzyme in a complicated manner. Some coenzyme is tightly bound as a prosthetic group\(^4\) to the apoenzyme, whereas loosely bound pyridoxal phosphate, which may be removed by dialysis\(^4\), is also present. As a rule, some pyridoxal phosphate is added to the organ extracts to increase the decarboxylase activity.

Inhibition of dopa decarboxylase may proceed by different mechanisms\(^5,6\). The loosely bound (or added) pyridoxal phosphate can be withdrawn from the system by an optically nonspecific binding by carbonyl trapping agents such as hydroxylamine and hydrazide, which react with the aldehydic group of the pyridoxal phosphate. On the other hand the substrate (L-dopa) may be displaced from the enzyme by an optically specific action of an inhibitor whose structure
is related to that of L-dopa. In the action of certain inhibitors both mechanisms may be involved. The inhibitory activity of amino acids and amines structurally related to dopa may be also due to the ability of the free amino group to bind the free pyridoxal phosphate in the form of a Schiff base, which may yield a tetrahydroisoquinoline derivative by cyclization.

An example of an inhibitor the action of which is based on both mechanisms is DL-α-methyldopa (see also chapter 1). Pütter and Kroneberg found that the inhibitory activity of the L-isomer is much more pronounced than that of the D-isomer. Only the L-isomer is active as a result of its interaction with apoenzyme and bound coenzyme while the inhibition resulting from the optically nonspecific binding of the free pyridoxal phosphate is of low magnitude (Lovenberg and Sourkes).

In addition to the above literature reviews on the inhibition of dopa decarboxylase, reference may also be made to the reports by Clark, Sourkes, Pletscher and Stone. The 6-alkyl-substituted DL-3,4-dihydroxyphenylalanines described in this thesis, were tested for their activity in vitro as inhibitors of dopa decarboxylase from guinea pig kidney; the results were compared with the activity of DL-α-methyldopa.

\[
\begin{align*}
\text{R=methyl (Me)} \\
\text{ethyl (Et)} \\
\text{isopropyl (IP)} \\
\text{tert-butyl (tB)}
\end{align*}
\]

5.2. METHOD OF DETERMINATION OF INHIBITORY ACTIVITY IN VITRO

Radioactive substrate is used in the method of Aures and Clark and Parulkar to measure in vitro inhibition of dopa decarboxylase. Especially in measuring minor differences in inhibitory activities more accurate results are obtained than when the manometric Warburg technique is employed (Hartman, Ferrini, etc.). The method of Aures and Clark involves the determination of the inhibition by measuring the radioactivity of the released on decarboxylation of DL-dopa-\(^{14}\)COOH under the influence of dopa decarboxylase in the presence of the inhibitor. For this determination a liquid
scintillation spectrometer is employed. A rotating diffusion chamber, designed for these experiments, is used for the absorption of the released $^{14}\text{CO}_2$ in the counting medium. Experiments with $^{14}\text{C}$-labelled bicarbonate have revealed that the recovery of $^{14}\text{CO}_2$ ranges from 90 to 97%.

As the diffusion chamber of Aures and Clark was not at our disposal an attempt was made to have the decarboxylation take place in a conventional 15 ml Warburg flask and to trap the evolving $^{14}\text{CO}_2$ in a suitable reagent in the center well. Ethanolamine and phenethylamine, being preferred according to literature in liquid scintillation measurements, proved unsuitable for trapping $^{14}\text{CO}_2$ in our experiment because of their volatility.

In 1962 Buhler$^{17}$ reported that the radioactivity of $^{14}\text{CO}_2$ trapped on a strip of filter paper soaked in aqueous potassium hydroxide, can be measured by means of the liquid scintillation spectrometer after transferring the moist paper strip to an appropriate scintillation solution immediately after decarboxylation. The composition of this solution was found to affect the efficiency of the counting considerably, combinations with polar solvents giving the best results.

Wang$^{18}$ demonstrated that the counting efficiency for $^{14}\text{C}$-labelled compounds on filter paper amounts to 55% in a conventional scintillation solution, if the compounds were insoluble in this counting medium. That author found that the dimensions of the filter paper and its orientation in the counting vial hardly affected the results of the measurements.

Buhler$^{17}$ tested the reliability of trapping $^{14}\text{CO}_2$ in the Warburg flask by releasing $^{14}\text{CO}_2$ from $^{14}\text{C}$-labelled sodium carbonate, followed by counting the potassium hydroxide paper. The efficiency of this process, erroneously called "counting efficiency" by Buhler, could be calculated from the net counting rate (in cpm) of the paper and the initial radioactivity in the flask (in dpm).

The result was found to be reasonably constant (36.9-42.0%) over a wide range of initial radioactivities.

When a mixture with a fixed amount of radioactive material and a variable amount of unlabelled sodium carbonate was used, the efficiency was also constant and of the same order of magnitude.

Except for minor alterations Buhler's method was adopted to determine the inhibitory effect of the above dopa derivatives on dopa decarboxylase. The scintillation solution described by Bray$^{19}$ for counting aqueous solutions was used to measure the radioactivity. Details on the procedure followed are given below.
EXPERIMENTAL PART

Enzyme preparation. The required enzyme preparation was obtained by the method of Schales and Schales\textsuperscript{20}. The kidneys (7.7 g) from two guinea pigs were homogenized in 28 ml of ice-cold water at 0° for 4 min, using a Virtis-"23"-Homogenizer. After standing in the refrigerator for 1 hr, the mixture was centrifuged (4400 r.p.m.) for 15 min. The fluid was decanted and then freeze-dried for a few hr. The remaining powder was stored in vacuo over CaCl\textsubscript{2} in the refrigerator. In a period of 5 months no decrease was observed in the activity of the preparation.

Substrate. DL-dopa\textsuperscript{14}COOH (0.050 mC), purchased from N.V. Philips-Duphar, the Netherlands and 78.4 mg of unlabelled DL-dopa were dissolved in 40 ml of water to give a substrate solution containing 2 μmole (ca. 0.25 μC) of dopa per 0.2 ml. Radiochemical purity was checked by paper chromatography; scanning the chromatograms with a Berthold thin layer scanner LB 2720 did not reveal radioactive impurities.

Inhibitors. After drying the alkylidopa compounds over P\textsubscript{2}O\textsubscript{5} in vacuo at 80° for 18 hr, solutions in water were prepared just prior to the experiment.

Decarboxylation. The following amounts were successively pipetted into the separate compartments of the Warburg flask: 0.5 ml of the inhibitor in water into the reaction compartment, 0.1 ml of 8 N H\textsubscript{2}SO\textsubscript{4} into one of the side arms, 0.2 ml of substrate solution (see above) into the other side arm, 0.1 ml of a fresh 10% KOH solution in water into the center well, 1 mg of dopa decarboxylase dry powder and 5 μl of pyridoxal phosphate in 1.5 ml 0.1 M phosphate buffer (Sörensen, pH 6.9) into the reaction compartment. A strip of filter paper (2x3 cm) was shaped into a small cylinder and put in the center well. The Warburg flasks (number of eight) were connected to their manometers and then placed in the bath which was kept at 37°. Via the gas inlet tube N\textsubscript{2} was passed over for 10 min. Next the system was closed and the substrate in one of the side arms was transferred to the reaction compartment. The flasks were shaken for 30 min and subsequently the HgSO\textsubscript{4} was transferred from the other side arm to the reaction mixture. Then the flasks were shaken for an additional hour. After the flasks had been opened, the KOH paper was transferred to a polyethylene counting vial filled with 10 ml of Bray's cocktail (60 g naphthalene, 4 g 2,5-diphenyloxazole (PPO), 0.2 g 1,4-di-2-(5-phenyloxazolyl)benzene (POPOP), 100 ml methanol, 20 ml ethylene glycol and dioxane to make 1 litre). The center well was rinsed twice with 0.5 ml of Bray's cocktail from the counting vial. On counting the KOH papers remained in cylindrical shape on the bottom of the counting vial; after 24 hr 90-98% of the radioactivity was still bound to the KOH papers in the scintillation solution. A Packard-Tri-Carb Scintillation Spectrometer Model 3375 was employed for the counting. Two of the eight flasks were used for blank determinations (without inhibitor). The percentages of inhibition were calculated by means of the average of the blank values. To the six other flasks were added six different concentrations of the inhibitor under investigation. The choice of the amounts of the reactants was dictated by both literature references\textsuperscript{14, 16, 17} and the results of preliminary studies in which different quantities of enzyme preparation and pyridoxal phosphate had been used to find a favourable measuring range.

Under the above experimental conditions ca. 15% of the substrate was decarboxylated in the blank determinations.
5.3. RESULTS AND DISCUSSION

Check on the efficiency of the method

Following Buhler's procedure an experiment was carried out to verify the limits within which the amounts of evolving $^{14}$CO$_2$ could be varied while the measured counting rate of the potassium hydroxide paper would still be proportional to the loss of the radioactivity of the decarboxylation mixture. Table 5 lists the results of duplicate experiments with three different concentrations of the inhibitor DL-$\alpha$-methyldopa.

When the experiment was initiated the radioactivity in the reaction compartment was equal to that of 0.2 ml of DL-dopa-$^{14}$COOH solution. After decarboxylation the radioactivity of the reaction mixture was measured by a duplicate determination of the cpm of 0.3 ml of the mixture in Bray's cocktail. After corrections for quenching, these values were converted into the total radioactivity in the 2.3 ml solution present.

<table>
<thead>
<tr>
<th>Concentration (mole)</th>
<th>Radioactivity in KOH paper (cpm)</th>
<th>Inhibition (%)</th>
<th>Decrease of radioact. in the flask (dpm)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ca.0.6x10$^{-4}$</td>
<td>32517</td>
<td>59</td>
<td>66213</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>31519</td>
<td>60</td>
<td>62394</td>
<td>50.5</td>
</tr>
<tr>
<td>ca.0.2x10$^{-4}$</td>
<td>52070</td>
<td>35</td>
<td>107264</td>
<td>48.5</td>
</tr>
<tr>
<td></td>
<td>49816</td>
<td>36</td>
<td>93744</td>
<td>53.2</td>
</tr>
<tr>
<td>ca.0.6x10$^{-5}$</td>
<td>67352</td>
<td>14</td>
<td>131999</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>68069</td>
<td>14</td>
<td>129108</td>
<td>52.7</td>
</tr>
<tr>
<td>-</td>
<td>77261</td>
<td>-</td>
<td>147659</td>
<td>52.3</td>
</tr>
<tr>
<td></td>
<td>79256</td>
<td>-</td>
<td>147532</td>
<td>53.7</td>
</tr>
<tr>
<td>average</td>
<td></td>
<td></td>
<td></td>
<td>51.4</td>
</tr>
</tbody>
</table>

From table 5 it is apparent that the efficiency was reasonably constant under the conditions applied. Some 51% of the $^{14}$CO$_2$ released in the reaction, could actually be counted. The quantities of radioactive material used in the decarboxylation experiments, were sufficient to obtain reliable results at this level of efficiency.
Inhibition of dopa decarboxylase from guinea pig kidney by DL-6-alkyldopa in comparison with DL-α-methyldopa

Details on the experimental procedure are given in chapter 5.2 (experimental part); the results are shown in table 6. Each percentage of inhibition was measured three times, the intervals between the determinations ranging from a few days to some weeks.

The average percentages of inhibition were plotted versus the logarithm of the concentration of the inhibitor in figure 2. The concentration at 50% inhibition as best derived from the concentration-activity curves in figure 2, is shown in table 6.

**TABLE 6** Inhibition of guinea pig kidney dopa decarboxylase by α-methyl- and 6-alkyldopa.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent inhibition at indicated concentration of the compound (mole)</th>
<th>Molarity at 50% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/3x 10^-5</td>
<td>1/3x 10^-4</td>
</tr>
<tr>
<td>α-Me-dopa</td>
<td>9 23 47 70 86 95 - -</td>
<td>3.8x10^-5</td>
</tr>
<tr>
<td></td>
<td>11 22 48 71 88 95 - -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 24 48 71 89 96 - -</td>
<td></td>
</tr>
<tr>
<td>6-Me-dopa</td>
<td>- - 5 8 27 60 90 97</td>
<td>7.1x10^-4</td>
</tr>
<tr>
<td></td>
<td>- - 3 8 25 62 87 96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - 2 10 27 62 90 97</td>
<td></td>
</tr>
<tr>
<td>6-Et-dopa</td>
<td>- - 5 9 32 67 81 85</td>
<td>5.6x10^-4</td>
</tr>
<tr>
<td></td>
<td>- - 5 10 33 67 80 84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - 4 7 33 69 80 85</td>
<td></td>
</tr>
<tr>
<td>6-iP-dopa</td>
<td>- - 3 10 35 69 83 86</td>
<td>5.3x10^-4</td>
</tr>
<tr>
<td></td>
<td>- - 4 13 35 69 80 84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - 4 12 35 68 80 85</td>
<td></td>
</tr>
<tr>
<td>6-tB-dopa</td>
<td>- - 5 19 57 76 80 85</td>
<td>2.6x10^-4</td>
</tr>
<tr>
<td></td>
<td>- - 6 20 57 76 80 84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- - 8 21 60 77 82 85</td>
<td></td>
</tr>
</tbody>
</table>
The results summarized in table 6 and figure 2 indicate that in vitro all four 6-alkyl-dopa compounds under investigation inhibit dopa decarboxylase, though to a lesser extent than does α-methyldopa.

The literature⁸ states two different points of time for the addition of the inhibitor to the substrate when the Warburg technique is used to determine the activity of dopa decarboxylase inhibitors. For the inhibitory activity of α-methyldopa Sourkes⁹, who incubated the inhibitor with the enzyme preparation for 10–15 min before addition of the substrate, found a higher value than Clark⁹, who added inhibitor and substrate at the same time.

For a comparison of the above activities of 6-methyl- and α-methyldopa with those found by Hartman¹⁵ who added inhibitor and substrate simultaneously, some additional experiments were performed. In the latter the substrate together with the inhibitor were transferred from one side arm to the reaction compartment.

The experimental conditions employed differed from those mentioned in chapter 5.2 in the following points: 0.2 ml of a solution of the inhibitor in water was pipetted into the side arm containing 0.2 ml of substrate; to 1.5 ml of the buffer solution of enzyme and pyridoxal phosphate in the reaction compartment was added 0.3 ml of water; after nitrogen had been passed over, substrate and inhibitor were added from the side arm.
The additional experiments were carried out in duplicate; for the results see table 7. It was not possible to investigate high concentrations of the inhibitor as a result of the poor solubility of the inhibitor in water (solubility decreases as the alkyl group becomes bulkier) and the small capacity of the side arm of the Warburg flask (max. 0.4 ml).

**TABLE 7** Inhibition of guinea pig kidney dopa decarboxylase by alkyl derivatives of dopa when inhibitor and substrate were added simultaneously to the enzyme preparation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent inhibition at indicated concentration of the compound (mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/3x10⁻²</td>
</tr>
<tr>
<td>α-Me-dopa</td>
<td>-</td>
</tr>
<tr>
<td>6-Me-dopa</td>
<td>40</td>
</tr>
<tr>
<td>6-Et-dopa</td>
<td>39</td>
</tr>
<tr>
<td>6-tP-dopa</td>
<td>40</td>
</tr>
<tr>
<td>6-tB-dopa</td>
<td>54</td>
</tr>
</tbody>
</table>

Table 7 shows that of the 6-alkyl compounds the tert. butyl derivative was again the most potent inhibitor; the three others showed few mutual differences. In comparison with the above values found on preincubation of inhibitor with enzyme preparation the inhibitory activity of all compounds had decreased. The decrease in activity of the 6-alkyl compounds was greater than that of the α-methyl compound. Simultaneous addition of inhibitor and substrate required a concentration of 6-methyldopa 33 times higher than that of α-methyldopa to obtain 50% inhibition; on preincubation of the inhibitor this factor was about 20.

**Discussion**

Alkyl substitution in the 6-position in dopa gives rise to compounds which, in vitro, slightly inhibit dopa decarboxylase from guinea pig kidney. Concentrations at 50% inhibition show a tendency for the inhibitory activity to increase as the alkyl group becomes bulkier.

The tert. butyl-substituted compound inhibits noticeably stronger than the methyl, ethyl and isopropyl derivatives.

Hartman$^{15}$ observed no inhibitory effect for 6-methyldopa but did find a weak
inhibition by $\alpha$-methyldopa. This discrepancy between Hartman's values and ours may be explained by the great difference in activity between the two compounds on simultaneous addition of inhibitor and substrate, as done by that author.

The extent to which the mechanism of action of the 6-alkyl compounds under investigation is comparable to that of $\alpha$-methyldopa cannot be inferred from the experimental values obtained. Pütter and Kroneberg$^6$) found L-$\alpha$-methyldopa to possess an inhibitory activity about 30 times stronger than D-$\alpha$-methyldopa at 50% inhibition and on preincubation of the inhibitor with enzyme-pyridoxal phosphate. On simultaneous addition of substrate and inhibitor and studying the time course of decarboxylation Pütter and Kroneberg found that the inhibition by the L-isomer started immediately whereas the inhibitory action of the D-isomer started slowly. Under these experimental conditions the D-isomer showed a much larger fall in activity than the L-isomer when they were compared with the values found on preincubation of the inhibitor.

The inhibitory activity of the 6-alkyl compounds described here, lay between that of L-$\alpha$-methyldopa and D-$\alpha$-methyldopa. The comparatively large reduction in activity by changing the experimental conditions regarding the addition of the inhibitor is a point of agreement between the 6-alkyl-substituted compounds and D-$\alpha$-methyldopa. Since, according to Pütter and Kroneberg$^6$), the activity (although low) of D-$\alpha$-methyldopa as an inhibitor results from binding of the free pyridoxal phosphate, the determination of the affinity of the 6-alkyl compounds for pyridoxal phosphate may contribute to an understanding of the mechanism of action.
LITERATURE

16. R. Ferrini and A. Glässer, Biochem. Pharmacol. 13, 798 (1964)
20. O. Schales and S. S. Schales, Arch. Biochem. (Biophys.) 24, 83 (1949)
SUMMARY

According to the literature, introduction of an alkyl substituent into the \( \alpha \)-position of 3,4-dihydroxyphenylalanine (dopa) gives rise to compounds which can affect the biosynthesis of catecholamines. In order to establish whether alkyl substitution in the 6-position of dopa would lead to comparable results, 6-methyl-, 6-ethyl-, 6-isopropyl- and 6-tert. butyldopa were synthesized.

The methyl, ethyl and isopropyl derivatives were prepared via the corresponding unsaturated azlactones (4-arylidene-2-phenyl-2-oxazolin-5-ones), which were obtained from 6-alkylveratraldehydes and hippuric acid according to Erlenmeyer-Plöchl.

Both nitration and bromination of 4-tert. butyveratrole produced a compound substituted in 5-position. The 1,2,4,5 substitution pattern was established by conversion of the products into derivatives already described in the literature and by NMR spectroscopy. The Grignard compound of 4-bromo-5-tert. butyveratrole gave 6-tert. butyveratraldehyde, which could be converted only in low yield into the corresponding azlactone by means of hippuric acid. By an 1,4-addition reaction of the Grignard reagent to 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one a mixture of the same tert. butyl-substituted azlactone and the ethyl ester of the corresponding \( \alpha \)-benzamidocinnamic acid was obtained. The ethyl ester presumably resulted from a nucleophilic attack by the leaving ethoxide ion at the carbonyl carbon atom of the azlactone ring.
Hydrogenation of the tert. butyl-substituted α-benzamido-dimethoxycinnamic acid, obtained by treatment of the azlactone-ester mixture with a base, yielded the corresponding dihydrocinnamic acid. Subsequent removal of the protecting groups (N-benzoyl and O-methyl) could not be effected using hydrohalogen acids without the tert. butyl group splitting off. The synthesis of 6-tert. butyldopa was eventually accomplished by successively replacing the benzyol group in the above dihydrocinnamic acid with a phthaloyl group, demethylation of the arylmethyl ethers in the N-phthaloyl derivative with boron tribromide and splitting off the phthaloyl group with hydrazine.

There is a conflict in the literature regarding the geometric configuration of the stable azlactone, which up till now has been the only isomer to be isolated in the Erlenmeyer-Plöchl reaction of benzaldehyde with hippuric acid.

A further investigation into the cis-trans assignment to substituted and unsubstituted azlactones was carried out on the basis of the NMR spectra of the geometric isomers of α-benzamido-3,4-dimethoxycinnamate and their related azlactones. The determination of the magnitude of the α-benzamido substituent effect on the signal of the olefinic proton in cis- and in trans-position in acrylic acid derivatives enabled the chemical shift of $H_8$ in the two benzamidocinnamates to be calculated. From the good agreement between the values calculated and the values found it could be concluded that the trans configuration should be assigned to the isomer derived from the stable azlactone. The trans assignment which was supported by additional data from the NMR spectra, was in contrast with a recent literature report. The fact that azlactones retain their configurations on conversion into the corresponding esters accounts for the assignment of the trans structure to the stable azlactone as well. This implies that the dimethoxyphenyl ring is trans to the carbonyl group.

The NMR data of the alkyl-substituted azlactones which were obtained in the synthesis of 6-alkyl-dopa, and of the derived cinnamates could be correlated with those of the non-alkyl-substituted trans compounds.

The trans configuration - also assigned to the alkyl-substituted azlactones - might be found for other azlactones, described in literature and prepared according to Erlenmeyer-Plöchl.

In conclusion the 6-alkyl-substituted dihydroxyphenylalanines were tested as inhibitors of dopa decarboxylase in vitro. DL-dopa-14COOH was decarboxylated in Warburg flasks under the influence of a dopa decarboxylase preparation.
from guinea pig kidney and in the presence of the inhibitor. The radioactivity of the released $^{14}\text{CO}_2$, which was trapped on potassium hydroxide paper could be determined in a suitable counting medium, using a liquid scintillation spectrometer. It appeared that in vitro all four 6-alkyl derivatives of DL-dopa are weaker inhibitors than DL-$\alpha$-methyldopa. As the alkyl substituent increased in bulk, the inhibitory activity showed a slight increase; introduction of the 6-tert. butyl group was found to raise inhibition noticeably.
SAMENVATTING

Invoering van een alkyl substituent op de α-plaats in 3,4-dihydroxyphenylalanine (dopa) levert volgens de literatuur verbindingen, die in staat zijn invloed uit te oefenen op de biosynthese van catecholamines. Om te kunnen onderzoeken of alkyl substitutie op de 6-plaats in dopa tot een vergelijkbaar resultaat leidt, werd de synthese uitgevoerd van 6-methyl-, 6-ethyl-, 6-isopropyl- en 6-tert. butyldopa.

Zowel het methyl, het ethyl, als het isopropyl derivaat kon verkregen worden via het corresponderende onverzadigde azlacton (4-arylideen-2-phenyl-2-oxazoline-5-on), dat bereid werd uit het 6-alkylveratraldehyde en hippuurzuur volgens Erlenmeyer-Plöchl.

Zowel nitrering als bromering van 4-tert. butyveratrool leverde een op de 5-plaats gesubstitueerde verbinding. Het 1,2,4,5-substitutiepatroon werd vastgesteld zowel door omzetting van de producten in derivaten die reeds beschreven waren in de literatuur, als met behulp van de NMR spectra. Het 4-broom-5-tert. butyveratrool leverde via de Grignard verbinding 6-tert. butyveratraldehyde, dat echter alleen in lage opbrengst met hippuurzuur tot het corresponderende azlacton kon worden omgezet.

1,4-Additie van de Grignard aan 4-ethoxymethyleen-2-phenyl-2-oxazoline-5-on resulteerde in de vorming van een mengsel van het zelfde tert. butyl-gesubstitueerde azlacton en de ethyl ester van het corresponderende α-benzamidokaneelzuur. Nucleofiele aanval van het "leaving" ethoxide ion op het carbonyl koolstof atoom van de azlacton ring was de vermoedelijke oorzaak van het ontstaan van genoemde ethyl ester.
Hydrogeneren van het tert. butyl-gesubstitueerde α-benzamido-dimethoxykaneelzuur, verkregen door behandeling van het azlacton-ester mengsel met een base, gaf het corresponderende dihydrokaneelzuur; het vervolgens verwijderen van de beschermende groepen (N-benzoil en O-methyl) met halogeenvoerverstof zuren bleek niet mogelijk zonder dat tegelijkertijd de tert. butyl groep afspaltte. Door achtereenvolgens in het bovenstaande dihydrokaneelzuur de benzoil groep te vervangen door een phtaloyl groep, de aryl-methyl ethers in het N-phtaloyl derivaat te demethyleren met boriumtribromide en vervolgens de phtaloyl groep af te splitsen met hydrazine, kon uiteindelijk de synthese van 6-tert. butyldopa gerealiseerd worden.

Er heerst in de literatuur geen eenstemmigheid over de geometrische configuratie van het stabiele azlacton, dat tot dusver als enige isomeer bij de Erlemeyer-Plöchl reactie van benzaldehyde met hippuurzuur geïsoleerd kon worden.

Met behulp van de NMR spectra van de geometrische isomeren van α-benzamido-3,4-dimethoxykaneelzuur ethyl ester en de beide afgeleide azlactonen werd de cis-trans toekenning aan al dan niet gesubstitueerde azlactonen nader onderzocht. Door het vaststellen van de grootte van het effect van een α-benzamido substituent op het signaal van het olefinisch proton in cis- en trans-positie in acrylzuur derivaten kon de chemische verschuiving van $H_B$ in beide α-benzamido kaneelzuur esters berekend worden. De goede overeenkomst tussen berekende en gevonden waarden leidde tot toekenning van de trans configuratie aan de ester die verkregen was uit het stabiele azlacton.

Deze structuur bepaling, die ondersteund werd door aanvullende gegevens uit de NMR spectra, was tegengesteld aan een desbetreffende uitspraak in recente literatuur. Op basis van het behoud van geometrische configuratie bij omzetting van azlactonen in de corresponderende esters, werd tevens aan het stabiele azlacton de trans structuur toegekend, d.w.z. de dimethoxyphenyl ring staat trans ten opzichte van de carbonyl groep.

De NMR gegevens van de, bij de synthese van 6-alkylidopa verkregen, alkyl-gesubstitueerde azlactonen en de daaruit verkregen kaneelzuur esters bleken goed aan te sluiten bij die van de niet-alkyl-gesubstitueerde trans verbindingen. Het is te verwachten dat de - eveneens voor de alkyl-gesubstitueerde azlactonen vastgestelde - trans configuratie toegekend kan worden aan meerdere in de literatuur beschreven azlactonen, die bereid zijn volgens Erlemeyer-Plöchl.
Tenslotte werden de 6-alkyl-gesubstitueerde dihydroxyphenylalanines getest op hun werking in vitro als remmer van dopa decarboxylase. DL-dopa$^{14}$COOH werd onder invloed van een dopa decarboxylase preparaat uit cavia nieren in aanwezigheid van de remmer in Warburg vaatjes gedecarboxyleerd. De radioactiviteit van het vrijkomende $^{14}$CO$_2$, dat met behulp van kaliumhydroxide papier werd gebonden, kon in een geschikt telmedium bepaald worden met een vloeistof-scintillatie-teller. De vier 6-alkyl derivaten van DL-dopa bleken in vitro zwakkere remmers te zijn dan DL-$\alpha$-methyldopa. Enige toename van de remmende activiteit werd gevonden bij toenemende omvang van de alkyl substituënt; 6-tert. butyldopa vertoonde duidelijk de sterkste werking.
STELLINGEN

I

De juistheid van Kochetkov's toekenning van de cis of trans structuur aan de onverzadigde azlactonen, verkregen uit erythro- en threo-N-benzoyl-3-phenylserine door eliminatie reacties, wordt door Filler in twijfel getrokken zonder dat daarvoor overtuigende argumenten worden aangevoerd.

Dit proefschrift, hoofdstuk 4.

II

De uitspraak van Galantay dat de methanolyse van 4-[2-acetoxy-1-(acetoxy-methyl)ethylideen]-2-phenyl-2-oxazoline-5-on onder vorming van een γ-methoxycrotonzure ester, waarschijnlijk via een intramoleculaire substitutie reactie verloopt, berust op a priori aangenomen onzekere gronden.

Dit proefschrift, hoofdstuk 4.

III

Dat de door Lovenberg voorgestelde verandering van de naam dopa decarboxylase in aromatische L-amino zuur decarboxylase een nauweuriger beschrijving van de substraatspecificiteit van dit enzym inhoudt, is aanwechtaar.


IV

Ten onrechte heeft Dat-Xuong aangenomen dat bij de alkylering van guajacol met tertiaire alcohollen onder invloed van boriumtrifluoride vrijwel uitsluitend het 4-tert. alkylguajacol ontstaat.


V

De selectiviteit van de test op ortho-dihydroxy verbindingen met phloroglucinol en natriumhydroxyde op filterpapier is twijfelachtig.

VI
De door Brunow vermelde NMR spectra van gesubstitueerde dibenzylideenbarnsteenuuranhydrides en hun fotoisomeren zijn onjuist geïnterpreteerd. De beschreven correlatie van de chemische verschuiving van het olefinisch proton met de berekende ladingsdichtheid op het corresponderende onverzadigde koolstof atoom kan nauwelijks de geometrische structuur toekennen ondersteunen.


VII
Electriciteitsvoorziening kan geschieden door aanvoer vanuit een electriciteitscentrale en door ter plaatse opwekken. Het is verheugend te constateren dat de laatste tijd in Nederland reëele aandacht wordt geschonken aan de laaatst genoemde mogelijkheid.

VIII
Ambtsdragers in de Gereformeerde Kerk, speciaal belast met jeugdwerk, dienen gekozen te worden door zowel de jongere belijdende- als doopleden.

IX
Het voorstel van de hoofdbesturen van de samenwerkende politieboden betreffende een nadere regeling van de bevoegdheden van de politie bij het handhaven van de openbare orde lijkt alleszins aanvaardbaar. Daarentegen kan de suggestie, de bestaande bewapening uit te breiden met onaangename sensatie verwekkende middelen, in strijd geacht worden met de intentie van het bij het Protocol van Genève (1925) verboden gebruik van chemische wapens.

De Politie, Orgaan van de Nederlandse Politiebond, jaargang 24, nr. 12, blz. 3 (1969).

X
Het verdient aanbeveling het aanbrengen van apparatuur voor koolmonoxyde detectie in auto's wettelijk te verplichten.

XI
In recente publicaties in de pers over toepassing van L-dopa in de therapie van de ziekte van Parkinson wordt ten onrechte geen rekening gehouden met het feit dat de naleving van de norm gesteld aan het registreren van een geneesmiddel, niet beïnvloed mag worden door de waarde die de patient aan dit geneesmiddel meent te moeten toekennen.

A. P. Morgenstern
Amsterdam, 14 november 1969