perature. To the residue was added 51 g. (0.37 mole) of N-carbethoxypyrrole and the mixture was allowed to stand at 0° for three days and room temperature for an additional day, at which time gas evolution had ceased. Ether was added, the solution was washed with dilute sodium carbonate and then steam distilled until four liters of distillate was collected. The distillate was extracted with four 250-ml. portions of ether and the ether extracts were dried and distilled giving recovered N-carbethoxypyrrole at 100° (50–55 mm.) and 2.5 g. of N-carbethoxy-3,2′-nornicotyrine at 141–143° (2 mm.). Similar extraction of the steam distillation residue gave an additional 0.5 g.; total, 3.0 g., 23% yield.

The picrate was prepared with ethanolic picric acid and recrystallized from ethanol, m.p. 146–147°.

Anal. Calcd. for $C_{18}H_{15}O_9N_5$: C, 48.5; H, 3.4; N, 15.7. Found: C, 48.6; H, 3.3; N, 15.7.

3,2′-Nornicotyrine.—To a solution of 1.3 g. of sodium hydroxide in 2.6 ml. of water was added 2.7 g. of the N-carbethoxy compound and sufficient ethanol to make a homogeneous solution. After standing at room temperature overnight, the solution was freed from ethanol by concentration at reduced pressure, the residue was extracted with three 50-ml. portions of ether, and the combined ether extracts were dried over magnesium sulfate and evaporated. Distillation of the residue gave 0.9 g. (50%) of 3,2′-nornicotyrine, b.p. 146–147° (1 mm.), which solidified in the receiver and melted at 98–100° on crystallization from benzene (5 ml.)–petroleum ether (1 ml.) (m.p. variously reported[9,15] from 99 to 101°).

Addition of saturated ethanolic picric acid gave a picrate which was crystallized from ethanol, m.p. 203–205° dec. (reported[9,15] m.p. from 202 to 204°).

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An Anomalous Result of an Attempted Dakin Reaction

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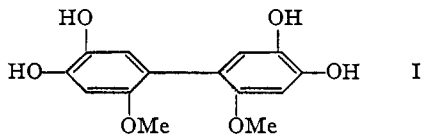
In connection with some studies being made in these laboratories, the synthesis of 4-methoxycatechol was undertaken. Kvalnes[1] reported, without giving any details of the synthesis, that he had obtained 4-methoxycatechol, m.p. 48–50°, in good yield by means of the Dakin[2] reaction, *i.e.*, replacement of the aldehydo or aceto group in ortho or para phenolic aldehydes or ketones with a hydroxyl group by means of hydrogen peroxide. When we added hydrogen peroxide to an aqueous solution of 2-hydroxy-5-methoxybenzaldehyde and sodium hydroxide, a precipitate was formed which softened at 200° and carbonized at 300°. Changing the reaction temperature from room temperature to 45°, and providing a nitrogen atmosphere to exclude oxygen, did not affect the results. The experiment was repeated twice, using lithium hydroxide and potassium hydroxide, respectively, and it was observed that, regardless of the hydroxide used, if the reaction mixture were allowed to stand for several days, the same precipitate was the only isolable product. Its solubility in hot water is compatible with the assumption that it is a polyhydroxy compound, but the high melting point of the material (m.p. 267–270° dec.) and its insolubility in ether suggest that it is not a simple nuclearly substituted catechol.

Raudnitz[3] has reported that the reaction between potassium persulfate and *p*-cresol yields 2,2′-dimethyl-5,5′-dihydroxydiphenyl, and Burton and Hopkins[4] have found that the oxidation of 4-methylcatechol by ferric acetate or silver oxide yields 4,5,4′,5′-tetrahydroxy-2,2′-dimethyldiphenyl and further oxidation products. These two analogous reactions suggest that the substance is a bis-(4-methoxycatechol) such as I or an isomer.



I

In an attempt to isolate the previously reported 4-methoxycatechol, a large quantity of the sodium salt of 2-hydroxy-5-methoxybenzaldehyde was treated with hydrogen peroxide according to Surrey's[5] procedure for making 3-methoxycatechol. Only a trace of a compound having the m.p. (48–50°) of 4-methoxycatechol[1] was found.

Experimental

2-Hydroxy-5-methoxybenzaldehyde.—This compound (b.p. 129° (12 mm.), lit. 124° (12 mm.)) was prepared by the Reimer–Tiemann reaction from *p*-methoxyphenol, as reported by Rubenstein.[6] The semicarbazone melted at 224–225°.

Bis-(4-methoxycatechol).—To a solution of 3.04 g. (0.02 mole) of 2-hydroxy-5-methoxybenzaldehyde in 20 ml. of 1 *N* KOH at room temperature was added in one portion 3.3 g. of 26% hydrogen peroxide in 30 ml. of water. The mixture became gradually brown, then cherry red, and was then heated for 15 minutes on a water-bath at 60°. The mixture was allowed to cool and stand overnight, whereupon 0.83 g. of precipitate was obtained. The precipitate was recrystallized three times from water slightly acidified with hydrochloric acid, m.p. 267–270°. When the mother liquor was allowed to stand several more days additional precipitate was isolated.

Anal. Calcd. for $C_{12}H_4(OCH_3)_2(OH)_4$: C, 60.4; H, 5.04; OCH_3, 22.3; mol. wt., 278; active H, 1.44. Found: C, 60.3, 59.8; H, 5.1, 5.1; OCH_3, 21.9; mol. wt.,[7] 279.6; active H, 1.46.

The same procedure with 1 *N* lithium hydroxide solution gave a similar result and a cleaner product.

4-Methoxycatechol.—To a mixture of 25 g. of 2-hydroxy-5-methoxybenzaldehyde and 83 ml. of 2 *N* sodium hydroxide at room temperature, 25 g. of 26% hydrogen peroxide in 145 ml. of water was added dropwise. The solution darkened and by the end of the addition, the temperature had risen to 60°. After the reaction mixture cooled, sodium chloride was added and the solution was extracted with chloroform. On standing the aqueous layer yielded several crops of the high-melting compound reported in this work. The solvent was removed from the chloroform extract and the residue was extracted with boiling water. A white precipitate, melting at 258–262°, which was evidently more of the biphenyl compound, formed from the extract overnight. The mother liquor was stored in the refrigerator and after several weeks a few large translucent crystals formed. This precipitate was filtered, dissolved in hot benzene, and reprecipitated with petroleum ether. The compound was yellow and melted at 49–51° (lit. 48–50°).

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(2) H. Dakin, *Am. Chem. J.*, **42**, 477 (1909).

(3) H. Raudnitz, *Ber.*, **63B**, 517 (1930).

(4) H. Burton and H. Hopkins, *J. Chem. Soc.*, 2445 (1952).

(5) A. Surrey, *Org. Syntheses*, **26**, 90 (1946).

(6) L. Rubenstein, *J. Chem. Soc.*, **127**, 1998 (1925).

(7) Determined by modification of the method of Signer (*Ann.*, **478**, 246 (1930)), the details of which were reported by the Analytical Branch of the Chemical Corps Chemical and Radiological Laboratories, Army Chemical Center, Maryland (Abstracts of Papers, 123rd Meeting American Chemical Society, March,, 1953, p. 5B).

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Optical Rotation of Peptides. VII. α- and γ-Dipeptides of Glutamic Acid and Alanine[1]

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Previous papers in this series[2] dealt with the synthesis and specific rotations of a number of alanine and lysine peptides. In this paper, the synthesis and specific rotations (in 0.5 *N* HCl) of eleven isomeric dipeptides containing glutamic acid (symbol: H·Glu·OH)[3] and alanine (H·Ala·OH)[3] are presented. Detailed data on the residue rotations[4] of glutamic acid and alanine residues in these peptides will be reported subsequently.

The dipeptides were prepared by coupling the appropriate N-carbobenzyloxyamino acid with amino acid benzyl esters according to the method of Boissonnas[5]; the resulting N-carbobenzyloxy dipeptide benzyl esters were reduced to the free peptides with palladium and hydrogen. Alanine peptides (H·Ala-Glu·OH) were obtained by coupling Z·Ala·OH with glutamic acid dibenzyl ester[6] (H·Glu·OBz). Glutamic acid α-peptides were
└OBz
synthesized from the carbobenzyloxy γ-benzyl ester[7] (Z·Glu·OH) and the γ-peptides from the
└OBz
carbobenzyloxy α-benzyl ester[6] (Z·Glu·OBz).

In the Van Slyke carboxyl nitrogen determination (ninhydrin),[8] γ-dipeptides of glutamic acid should yield 1 mole of COOH nitrogen (COOH, N), while the α-peptides should yield none. That this is the case for these two types of glutamic acid dipeptides, synthesized by the methods outlined above, can be seen from Table II (compounds 15–22).

In the Van Slyke amino nitrogen determination (nitrous acid), the α-peptides give correct analytical values (1 mole of amino N, *cf.* Table II, compounds 12–18). With the γ-peptides, both amino and peptide nitrogens react (2 moles of amino N, Table II, compounds 19–22). This observation constitutes an important distinction between α- and γ-peptides of glutamic acid. The underlying mechanism will be discussed elsewhere in connection with additional data.[9]

The purity of these peptides was further confirmed by chromatography; α- and γ-isomers are readily separable by this method (*cf.* Table II, column R_{Glu}).

It has been established[10] that the synthesis of dipeptides of glutamic acid *via* its carbobenzyloxy anhydride (Z·Glu·O),[11] yields mixtures of α- and γ-
└──┘
peptides. It was thought that the synthesis of pure γ-peptides could be accomplished *via* the γ-azide of carbobenzyloxyglutamic acid, (Z·Glu·OH)[10,12–14].
└N₃
However, we have found[15] that this procedure is not unequivocal, but leads to mixtures of α- and γ-peptides; from these mixtures, pure α- and γ-peptides can sometimes be obtained by fractional crystallization.[15]

In view of the difficulties encountered in the preparation of γ-peptides, it is essential that, in every case, homogeneity be established by all of the analytical procedures described above.

Experimental[16]

Starting Materials.—The syntheses and properties of some of the starting materials have been previously described: L- and D-alanine,[17] H·Ala·OBz (L) and (D) (ref. 17, compounds 5, 6), L- and D-glutamic acid,[6] H·Glu·OBz (L) and
└OBz
(D), and Z·Glu·OBz (L) (ref. 6, compounds 1, 2 and 5). Other starting materials used were: Z·Ala·OH (L) and (D),[11] Z·Glu·OH (L)[7] and H·Glu·OH (L)[7] (carboxyl nitrogen[8]
└OBz └OBz
content (ninhydrin, 100°, 7 min., *p*H 2.5): Calcd. for $C_{12}H_{15}O_4N$ (237.2): carboxyl N, 5.9. Found: carboxyl N, 5.9).

Carbobenzyloxy Dipeptide Benzyl Esters (Compounds 1–11).—The free COOH group of the carbobenzyloxyamino acids (Z·Ala·OH, Z·Glu·OH, Z·Glu·OBz) is converted into
└OBz
a tertiary amine salt. The tertiary amine salts, in turn, are converted with ethyl chlorocarbonate into the mixed an-

(1) From a dissertation to be submitted by Howard Sachs in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University. Erwin Brand deceased July, 1953.

(2) Paper VI, E. Brand, B. F. Erlanger and H. Sachs, THIS JOURNAL, **74**, 1851 (1952).

(3) The following abbreviations and symbols are used (*cf.* E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946); ref. 2, Table I, footnote *a*; ref. 6, footnote 2): Z, carbobenzyloxy, $C_6H_5CH_2OCO$; Bz, $C_6H_5CH_2$; Ala, $NHCH(CH_3)CO$, C_3H_6ON; Glu, $NHCH(CH_2CH_2COOH)CO$, $C_5H_7O_3N$; peptide linkage indicated by dash, –; configuration follows compound in parentheses, ( ). When the γ-carboxyl group of glutamic acid is substituted, the following symbol is used for the residue: Glu. *e.g.*, N-carbobenzyloxy-L-alanine benzyl ester,
└OH
Z·Ala·OBz (L); N-carbobenzyloxy-D-glutamic acid γ-benzyl ester, Z·Glu·OH (D); N-carbobenzyloxy-L-alanyl-D-glutamic acid dibenzyl
└OBz
ester, Z·Ala-Glu·OBz (LD); N-carbobenzyloxy-α-benzyl γ-L-glutamyl-
└OBz
D-alanine benzyl ester, Z·Glu·OBz (LD); γ-L-glutamyl-D-glutamic acid,
└Ala·OBz
H·Glu·OH (LD); α-L-glutamyl-L-alanine, H·Glu-Ala·OH (2L)
└Glu·OH

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(9) H. Sachs and E. Brand, unpublished work.

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(14) B. Hegedus, *Helv. Chim. Acta*, **31**, 737 (1948).

(15) H. Sachs and E. Brand, Am. Chem. Soc., Los Angeles Meeting, March, 1953, Abstracts p. 30c; H. Sachs and E. Brand, *Federation Proc.*, **12**, 262 (1953).

(16) We are indebted for analytical work to T. Zelmenis (total and amino N).

(17) B. F. Erlanger and E. Brand, THIS JOURNAL, **73**, 3508 (1951).