103. *Autoxidation of Polyphenols. Part I. Autoxidation of Methyl Gallate and its O-Methyl Ethers in Aqueous Ammonia.*

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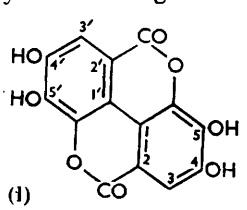
Autoxidation of methyl gallate in 2N-ammonia, which eventually affords ellagic acid, has given a polymer, hydrogen peroxide (trace), gallic acid, and a derivative of 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid. The hexamethoxydiphenic acid was prepared by the Ullmann synthesis, as well as from 4 : 5 : 6 : 4′ : 5′ : 6′-hexabenzyloxydiphenic acid.[1] Analogous autoxidation of methyl 3-*O*-methylgallate afforded 4 : 4′-di-*O*-methylellagic acid.

Although ellagic acid (I) has been prepared by aeration of an ammoniacal solution of ethyl gallate,[1-3] the reaction has received little attention, but Erdtman[4] obtained low yields of ellagic acid on autoxidation in dilute solutions of barium hydroxide, sodium hydrogen carbonate, or disodium hydrogen phosphate. He also found that no ellagic acid was formed when gallic acid was autoxidised in these solutions.



(I)

In the present study, the reaction of methyl gallate had been investigated by manometry, chromatography, and by the use of model substances.

Methyl 4-*O*-methylgallate, methyl 3 : 4-di-*O*-methylgallate, and methyl syringate were recovered unchanged from the autoxidation reaction, but methyl 3-*O*-methylgallate was converted in small proportion into 4 : 4′-di-*O*-methylellagic acid, characterised as the crystalline diacetate. 4 : 4′-Di-*O*-methylellagic acid affords colour reactions, fluorescence in ultraviolet light, R_F values, and a lactonic band at 1712 cm.$^{-1}$, consistent with the behaviour of the related ellagic and flavellagic acid.[5]

Two vicinal hydroxyl groups, correctly oriented with respect to the alkoxycarbonyl substituent, are therefore essential if autoxidation is to afford ellagic acid derivatives. Since semiquinones are stabilised as anions in alkaline solution,[6] symmetrical dimerisation of semiquinone radicals is probably involved.[7] Hydrogen peroxide is associated with the oxidation by molecular oxygen of quinols to quinones in water,[8 9] and the correspondingly low proportion of residual hydrogen peroxide which accumulated during ellagic acid synthesis is consistent with dimerisation of semiquinone.[10] The oxygen balance of 0·93 mole per mole of methyl gallate is however too large to be commensurate with ellagic acid synthesis; but as only 60% of this substance is formed by autoxidation, the additional uptake is presumably associated with the accompanying formation of polymer. Early in the autoxidation, a red colour develops, suggesting the presence of an *o*-quinonoid compound (semiquinone), but the colour was too transient for the spectrum to be measured.

It has now been shown that no ellagic acid is produced by aeration of an ammoniacal solution of gallic acid, and it follows that in the corresponding autoxidation of methyl gallate dimerisation preceded hydrolysis of the ester group. It is therefore suggested that dimethyl 4 : 5 : 6 : 4′ : 5′ : 6′-hexahydroxydiphenate is an intermediate; and the formation of 2 : 3 : 4 : 2′ : 3′ : 4′-hexahydroxydiphenyl by autoxidation of pyrogallol in strong

[1] Schmidt, Voigt, Puff, and Köster, *Annalen*, 1954, **586**, 165.
[2] Herzig, Pollak, and von Bronneck, *Monatsh.*, 1908, **29**, 278.
[3] Reichel and Schwab, *Annalen*, 1942, **550**, 152.
[4] Erdtman, *Svensk kem. Tidskr.*, 1935, **47**, 223.
[5] Hathway, *Nature*, 1956, **177**, 747.
[6] Michaelis *et al.*, *J. Biol. Chem.*, 1938, **119**, 133; *J. Amer. Chem. Soc.*, 1937, **59**, 2460; 1938, **60**, 202, 214, 1667, 1678; Pauling, "The Nature of the Chemical Bond," Cornell Univ. Press, Ithaca, 1940, p. 276.
[7] Cf. Grimshaw, Haworth, and Pindred, *J.*, 1955, 833.
[8] Hathway and Seakins, *Nature*, 1955, **176**, 218.
[9] Beer, Broadhurst, and Robertson, *J.*, 1954, 1947.
[10] Siegel, *J. Amer. Chem. Soc.*, 1956, **78**, 1753.

alkali [11] is relevant. Accordingly, when a solution of methyl gallate was acidified after brief autoxidation and the solvent-extractable material was treated successively with diazomethane and methanolic potassium hydroxide, a small quantity of 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid was separated by chromatography from a big preponderance of tri-*O*-methylgallic acid. 4 : 5 : 6 : 4′ : 5′ : 6′-Hexamethoxydiphenic acid has previously been prepared by the prolonged treatment of tetra-*O*-methylellagic acid with hot sodium hydroxide solution and methyl iodide.[12] In the present study, 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid has been prepared in (1) 40% yield by the Ullmann synthesis in dimethylformamide starting from methyl 2-bromo-*O*-trimethylgallate,[13] and hydrolysis of the resulting ester, and (2) 90% yield from 4 : 5 : 6 : 4′ : 5′ : 6′-hexabenzyloxydiphenic acid,[1] by successive catalytic debenzylation, methylation, and hydrolysis. A small quantity of methyl tri-*O*-methylgallate, which was a by-product in the Ullmann synthesis, was removed from dimethyl 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenate by selective hydrolysis. Paper chromatography of tri-*O*-methylgallic and 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid was investigated: the successful separation by a dilute acetic acid solvent system was extendible to cellulose column chromatography.

Of the four selected methyl ethers of methyl gallate, methyl 3-*O*-methylgallate is new. It was prepared by Fischer–Speier methylation of the corresponding acid, which was synthesised *via* methyl 4 : 5-diphenylmethylenedioxy-3-hydroxybenzoate.[14] The parent acid was regenerated by hydrolysis of methyl 3-*O*-methylgallate, which was further characterised as diacetate and bis-3 : 5-dinitrobenzoate. The colour reactions of methyl 3-*O*-methylgallate with ferric chloride reagent accord with those for the corresponding acid.[15]

The autoxidation of gallic acid and methyl gallate in ammonia affords polymers which remain adsorbed near the top of magnesium aluminium silicate columns during development with phosphate buffer of pH 12. These polymers also migrate a short distance towards the anode during low-voltage ionophoresis on paper at pH 12. It has now been shown that methyl gallate is hydrolysed to gallic acid under the conditions of the autoxidation in approximately the same proportion as that in which the corresponding polymeric by-product is formed, but gallic acid can no longer be detected when autoxidation is complete. On account of this observation, and the known stability of an alkaline solution of ellagic acid to hydrogen peroxide at room temperature,[16] it is suggested that gallic acid may be the chemical precursor of the polymeric by-product.

Experimental

Solutions were evaporated in nitrogen at <35°. M. p.s were determined on a Kofler block. Paper chromatography was carried out at 22° ± 2° in all-glass apparatus. Chromatograms were dried in a current of warm air at 60°. A Hanovia mercury arc fitted with a Wood's-glass filter was used to examine chromatograms for fluorescent zones. Johnsen, Jörgensen, and Wettre's Solka Floc cellulose (Grade, BW200) was homogenised in acetone by a top-drive macerator, and the slurry was transferred to chromatography tubes. The columns were developed with acetone, stored for 24 hr., and developed under pressure with aqueous 10% acetic acid until free from acetone. Florex (Grade, XXS), a magnesium aluminium silicate, was washed with phosphate buffer (pH 12·0) before use. Peter Spence (Grade H) alumina was treated with 2N-acetic acid, then thoroughly washed with methanol, and reactivated (Brockmann, grade II/III) at 100°/12 mm.

Attempted Autoxidation of O-*Methyl Ethers of Methyl Gallate.*—Aeration of a solution of methyl syringate, 3 : 4-di-*O*-methylgallate, and 4-*O*-methylgallate (0·025 mole) respectively in

[11] Harries, *Ber.*, 1902, **35**, 2957.
[12] Herzig and Pollak, *Monatsh.*, 1908, **29**, 263.
[13] Feist and Dschu, "Festschrift für A. Tschirch," Leipzig, 1926, p. 28.
[14] Bradley, Robinson, and Schwarzenbach, *J.*, 1930, 793.
[15] Fischer and Freudenberg, *Ber.*, 1913, **46**, 1123.
[16] Haworth, Pindred, and Jefferies, *J.*, 1954, 3617.

2N-ammonia (100 ml.) for 24 hr., followed by acidification, afforded unchanged methyl syringate, m. p. 106°, and 3 : 4-di-*O*-methylgallate, m. p. 84°, in quantitative recovery. The 4-*O*-methylgallate, m. p. 147°, was recovered quantitatively by extraction with ethyl acetate. All three esters were identified by mixed m. p.s.

When a solution of methyl 3-*O*-methylgallate (9·9 g.) in 2N-ammonia (200 ml.) was aerated, a rose-coloured solution developed, and after 60 hr. a small precipitate was removed. Trituration with 12N-hydrochloric acid gave a precipitate (200 mg.), which was removed, washed free from mineral acid (Congo-red), and dried over potassium hydroxide at 20°/0·02 mm. for 20 hr. This crude product, which gave a weak Griessmayer colour reaction, was refluxed with acetic anhydride (50 ml.) containing 3 drops of 18N-sulphuric acid for 2 hr. When the chilled mixture was poured into ice-water, crude diacetate was recovered, and dried at 110° for 1 hr. Sublimation (3 hr.) at 300°/0·05 mm. gave of 4 : 4′-*di*-O-*methylellagic acid diacetate* (80 mg.), m. p. >320° (Found : C, 57·1; H, 3·6. $C_{20}H_{14}O_{10}$ requires C, 58·1; H, 3·4%). The diacetate in boiling pyridine (5 ml.) was treated with acetic acid (5 ml.) and 12N-hydrochloric acid (5 ml.), then refluxed for 2 hr. 4 : 4′-*Di*-O-*methylellagic acid* (50 mg.) was removed, washed free from mineral acid (Congo-red), and dried at 110° for 1 hr. It crystallised from *NN*-dimethylformamide as very pale yellow needles, m. p. >320° (Found : C, 57·8; H, 3·2. $C_{16}H_{10}O_8$ requires C, 58·2; H, 3·1%), which were dried at 110°/0·05 mm. The acid gave a weak Griessmayer colour reaction, an intense lemon solution in 2N-sodium hydroxide, and a violet fluorescence in ultraviolet light, which became yellow on treatment of the substance with ammonia. It had R_F 0·70 in aqueous 40% (v/v) formamide, buffered at pH 3·5 with formic acid.

Acidification of the original reaction filtrate gave an intractable resin.

Autoxidation of Methyl Gallate.—(1) Autoxidation of methyl gallate (10 g.) in 2N-ammonia (200 ml.) for 48 hr. afforded ellagic acid tetra-acetate (7·6 g., 60%) which crystallised from acetic anhydride (1200 ml.) as needles (7·5 g.), m. p. 344° (Found : C, 55·7; H, 3·3. Calc. for $C_{22}H_{14}O_{12}$: C, 56·1; H, 3·0%).

(2) Absorption of oxygen by methyl gallate (2 millimoles) in 2N-ammonia (20 ml.) was followed volumetrically in a hydrogenation apparatus. When it ceased (4 days), ammonium ellagate was removed and washed, and the combined filtrate and washings were acidified, and poured on an alumina column (10 × 1·5 cm.) prepared in water. Polymer was removed, and potassium iodide (10 g.) was added to the aqueous eluate. After storage for 5 min. in the dark, the liberated iodine was titrated with 0·01N-sodium thiosulphate. The results were : total O_2 uptake, 0·96; H_2O_2 formed, 0·05; O_2 balance, 0·93 mole per mole of methyl gallate. In a parallel experiment, the oxidising agent left in solution after removal of ellagic acid and polymer was shown to be hydrogen peroxide. Vacuum-distillation gave a colourless, aqueous solution which was spectroscopically indistinguishable from distilled water in the range 300—700 mμ and reacted positively with acidified potassium iodide and titanic sulphate.

(3) On addition of 1 drop of 15N-ammonia to an aqueous solution of methyl gallate (0·05 mmole/l.) a very transient crimson colour developed.

(4) Aeration of a 2N-ammonia (200 ml.) solution of methyl gallate (5 g.) for 30 min. gave a precipitate of ammonium ellagate which was removed, and the acidified filtrate was continuously extracted with dry ether for 5 hr. The dried ($MgSO_4$) ether extract (400 ml.) was chilled, treated with dried (KOH) ethereal diazomethane (*ca.* 5·6 g. in 200 ml.) at 0°, and kept at 0° for 16 hr., then excess of diazomethane was destroyed by acetic acid. Evaporation of the solvent left a syrup which was refluxed with N-methanolic potassium hydroxide (100 ml.). The solvent was evaporated, and an aqueous solution (25 ml.) of the residue was extracted with ether. Residual ether was removed *in vacuo* and the aqueous phase acidified with 12N-hydrochloric acid. The precipitate (1·25 g.) was removed, washed free from mineral acid (Congo-red) with ice-water, and dried at 110°. A glacial acetic acid solution (5 ml.) of this precipitate (300 mg.), m. p. 168—185°, was adsorbed on a cellulose column (21·5 × 2·5 cm.) prepared in and developed with 10% acetic acid. Initial eluate (77 ml.) was discarded, and thereafter eluate was automatically fractionated in 3 ml. portions. Spots were withdrawn and chromatographed (see below) on Whatman filter paper no. 1. 4 : 5 : 6 : 4′ : 5′ : 6′-Hexamethoxydiphenic acid (4 mg.), m. p. 240°, undepressed on admixture with a specimen, m. p. 243°, separated at 0° from fraction 5.

Autoxidation of Gallic Acid.—After gallic acid (4·25 g.) in 2N-ammonia (100 ml.) had been aerated for 72 hr., humic acid was precipitated by 12N-hydrochloric acid from the resulting dark solution.

Preparation of 4 : 5 : 6 : 4′ : 5′ : 6′-*Hexamethoxydiphenic Acid.*—(1) A dry (CaH_2) dimethylformamide solution (100 ml.) of methyl 2-bromotri-*O*-methylgallate [13] (10 g.) was refluxed with activated copper [17] (10 g.) for 8 hr., a second portion of metal (10 g.) being added after 4 hr. The mixture was poured into ice-water (2 l.), and the sediment centrifuged off and dried over phosphoric oxide at 20°/0·05 mm. for 20 hr. The supernatant liquid was evaporated, and evaporation was continued during the gradual addition of more water (2 l.) in order to remove the last traces of dimethylformamide. The product (6·8 g.) was recovered from both the sediment and the evaporation residue by extraction with ethyl acetate. A solution of the residue in benzene–light petroleum (b. p. 40—60°) (1 : 1; 50 ml.) was poured on an alumina column (20 × 2·5 cm.). Evaporation of the benzene–light petroleum (1 : 1) eluate left a syrup (6·1 g.), which was refluxed with 0·2N-methanolic potassium hydroxide (100 ml.) for 2 hr. The solvent was removed, and the residue acidified with 0·1N-hydrochloric acid, then the product was extracted with ethyl acetate. Evaporation of the dried ($MgSO_4$) solvent left a partially crystalline residue, which was extracted with boiling water (500 ml.). Oil was removed and, on evaporation, the filtrate deposited tri-*O*-methylgallic acid (1·2 g.), forming needles, m. p. and mixed m. p. 167°. The oil was dissolved in chloroform, which was washed successively with saturated sodium hydrogen carbonate solution and water. Evaporation of the dried ($MgSO_4$) solvent left dimethyl 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenate (4 g.) which crystallised in 14 days, forming rosettes, m. p. 95—97° (m. p. 90—95° and 110—111° have been recorded [12]) (Found: C, 58·6; H, 5·8. Calc. for $C_{22}H_{26}O_{10}$: C, 58·7; H, 5·8%). The ester (4 g.) was refluxed with N-methanolic potassium hydroxide (100 ml.) for 3 hr. Evaporation left a solid residue, which was dissolved in water (20 ml.) and acidified with 12N-hydrochloric acid (10 ml.). Crude 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid (2·7 g., 40%), m. p. 234—238°, was removed and washed free from mineral acid. 4 : 5 : 6 : 4 : 5′ : 6′-Hexamethoxydiphenic acid crystallised from ethanol as rhombs (2·6 g.), m. p. 243° (Found: C, 56·6; H, 5·6. Calc. for $C_{20}H_{22}O_{10}$: C, 56·9; H, 5·3%).

(2) A methanolic solution (40 ml.) of 4 : 5 : 6 : 4′ : 5′ : 6′-hexabenzyloxydiphenic acid [1] (1·18 g.) was hydrogenated at 1 atm., in the presence of 10% palladised charcoal, and the product was methylated with excess of diazomethane, then hydrolysed with N-methanolic potassium hydroxide. Crude 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid (490 mg., 90%; cf. 42% yield by Schmidt and Demmler [18]), m. p. 230—238°, was dried at 110° for 1 hr. A single sublimation at 220°/0·05 mm. afforded 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid (430 mg.), m. p. 243° (Found: C, 56·9; H, 5·3%).

Chromatographic Separation of 4 : 5 : 6 : 4′ : 5′ : 6′-*Hexamethoxydiphenic and Tri*-O-*methylgallic Acid.*—Marker spots (5 μl.) of 0·5% (w/v) solutions of the acids were applied to start-lines, 4 cm. from the lower edge of Whatman filter paper no. 1, of length 57 cm. Single-way ascending chromatography was effected with the following solvent systems: (1) benzyl alcohol–*tert.*-butyl alcohol–*iso*propyl alcohol–water (3 : 1 : 1 : 1, by vol.) containing 1·8% (w/v) of formic acid; [19] (2) the upper phase of equilibrated and esterified butan-1-ol–acetic acid–water (63 : 10 : 27, by vol.).[20] Papers were irrigated for 20 hr. The acids were detected by their fluorescence in ultraviolet light, and as yellow spots against a green background, when a solution of bromocresol-green at pH 5·5 was employed as a spraying reagent.[21]

	R_F in solvent-system		R_F on paper	
Acid	1	2	no. 1	no. 20
Tri-*O*-methylgallic	0·92	0·91	0·73	0·72
4 : 5 : 6 : 4′ : 5′ : 6′-Hexamethoxydiphenic	0·91	0·90	0·65	0·68

Marker spots were also applied to start-lines, 11 cm. from the upper edge of Whatman filter papers no. 1, and no. 20 previously washed chromatographically with N-acetic acid. Descending chromatography was effected with N-acetic acid, the papers being irrigated for 4 and 6 hr. respectively.

A glacial acetic acid solution (5 ml.) of 4 : 5 : 6 : 4′ : 5′ : 6′-hexamethoxydiphenic acid

[17] Ullmann, *Annalen*, 1904, **332**, 38; Kleiderer and Adams, *J. Amer. Chem. Soc.*, 1933, **55**, 4219.
[18] Schmidt and Demmler, *Annalen*, 1954, **586**, 179.
[19] Stark, Goodban, and Owens, *Analyt. Chem.*, 1952, **23**, 413.
[20] Campbell, Work, and Mellanby, *Biochem. J.*, 1951, **48**, 106.
[21] Lugg and Overell, *Nature*, 1947, **160**, 87.

(75 mg.) and tri-*O*-methylgallic acid (50 mg.) was adsorbed on a cellulose column (24 × 2·5 cm.) and the 10% acetic acid eluate was fractionated in 3 ml. tubes. The hexamethoxydiphenic acid crystallised at 0° in tubes 31—38, forming rhombs (55 mg.), m. p. 241°, undepressed by admixture with an authentic specimen, m. p. 243°.

Methyl 3-O-*Methylgallate*.—A cold solution of methyl 4 : 5-diphenylmethylenedioxy-3-hydroxybenzoate [14] (29·7 g.) in absolute methanol (100 ml.) was treated with dried (KOH) ethereal diazomethane (*ca.* 5·6 g. in 200 ml.) at 0°. After 1 day at 0°, prisms (16 g.) of methyl 4 : 5-diphenylmethylenedioxy-3-methoxybenzoate, m. p. 135° (lit.,[14] m. p. 135°), were removed, and further product (14 g.), m. p. 135°, was recovered from the mother-liquor (yield, 30 g., 100%). 3-O-*Methylgallic acid* crystallised from water, forming prismatic needles of hemihydrate, m. p. 225° (Found: C, 49·7; H, 4·6; loss at 110°/0·05 mm., 4·8. Calc. for $C_8H_8O_5,\frac{1}{2}H_2O$: C, 49·7; H, 4·7; H_2O, 4·7%), which were dried over phosphoric oxide at 60°/15 mm. A stream of hydrogen chloride was conducted through a boiling solution of this acid (14·1 g.) in absolute methanol (200 ml.) for 3 hr. Evaporation left a solid residue which was triturated with excess of sodium hydrogen carbonate solution and washed with ice-water. *Methyl* 3-O-*methylgallate* was recovered in the form of rhombs (12 g.), m. p. 116° (Found: C, 54·7; H, 5·2. $C_9H_{10}O_5$ requires C, 54·5; H, 5·1%), which were dried over phosphoric oxide at 60°/0·1 mm. for 4 hr. With ferric chloride an aqueous solution of the ester gave a bluish-black solution, whereas the alcoholic solution afforded a green solution.

Hydrolysis of methyl 3-*O*-methylgallate (100 mg.) with boiling N-methanolic potassium hydroxide (10 ml.) under nitrogen for 1 hr. afforded prismatic needles (60 mg.), m. p. 225°, undepressed on admixture with 3-*O*-methylgallic acid hemihydrate.

Methyl 4 : 5-*diacetoxy*-3-*methoxybenzoate* crystallised from ethanol–water (1 : 4) as lamellæ, m. p. 99° (Found: C, 55·6; H, 5·0. $C_{13}H_{14}O_7$ requires C, 55·4; H, 5·0%), which were dried over phosphoric oxide at 60°/0·2 mm. and gave no ferric reaction. *Methyl* 4 : 5-*bis*-(3 : 5-*dinitrobenzoyloxy*)-3-*methoxybenzoate* crystallised from acetic anhydride as needles, m. p. 213° (Found: N, 9·0. $C_{23}H_{14}O_{15}N_4$ requires N, 9·5%).

Hydrolysis of Methyl Gallate with Dilute Ammonia.—When a solution of methyl gallate (5 g.) in 2N-ammonia (200 ml.) was stored under hydrogen at 20° for 2 days, acidified with 12N-hydrochloric acid, and basified with excess of solid sodium hydrogen carbonate, unchanged methyl gallate was recovered by continuous extraction with ether. Continuous extraction of the acidified aqueous phase with ether afforded gallic acid (500 mg.), m. p. and mixed m. p. 240°. Gallic acid, m. p. 240°, R_F 0·43 and 0·72 in N-acetic acid and *sec.*-butyl alcohol–acetic acid–water (14 : 1 : 5), was isolated by similar means after 1 day's autoxidation of methyl gallate, but could not be chromatographically detected after 2 days' reaction.

Ionophoresis.—Gallic acid and methyl gallate (2 millimoles) were autoxidised for 2 days. Spots (5 µl.) of the solutions were then applied to a start-line, 10 cm. from the cathode end of a strip (33 × 10 cm.) of Whatman filter paper no. 3MM, previously moistened [22] with phosphate buffer of pH 12·0. Ionophoresis was carried out in a horizontal apparatus,[23] phosphate buffer of pH 12·0 being employed as chromatographic solvent; d.c. at 250 v and 40 mA was applied for 2 hr. The electrogram was dried at 100° for 15 min. In both cases, tan-coloured polymer migrated a short distance towards the anode, accompanied by blue and yellow fluorescent zones of substances which migrated correspondingly farther in the same direction.

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[22] Kunkel in Glick's "Methods of Biochemical Analysis," Interscience Publ. Inc., New York, Vol. I, 1954, p. 141; Linskens, "Papierchromatographie in der Botanik," Springer-Verlag, Berlin, 1955, p. 9.
[23] Grassmann and Hannig, *Z. physiol. Chem.*, 1952, **290**, 1.