990. *The Structure of Picrotoxic Acid.*

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The chemical relations between picrotoxic acid and picrotoxinin derivatives establish the functional groups, which are confirmed by infrared measurement. Methylation of methyl dihydro-*O*-methylpicrotoxate by a modified Purdie procedure, hydrolysis of methyl dihydrodi-*O*-methylpicrotoxate by hydrazine, and preparation of a picrotoxinin monoacetate are described. The present work in conjunction with Conroy's carbon skeleton [1] for the parent compound suggests two structures for picrotoxic acid, of which (I) is preferred.

In 1948, an investigation of the chemistry of picrotoxic acid and picrotoxinin was begun because, although the structure of several degradation products had been established,[2] these were obtained by reactions sufficiently drastic to obscure the structural relation to the parent compound.

Mercer and Robertson [2] had prepared a methylation product of dihydropicrotoxic acid to which the partial formula $Pr^{i}\cdot C_{11}H_{10}O_3(OMe)_2\cdot CO_2Me$ was given, but the reaction conditions were unsuited for preparative work. Further work on this and many related compounds has now established this partial formula for methyl dihydrodi-*O*-methylpicrotoxate. Methylation of dihydropicrotoxic acid by methyl sulphate and potassium carbonate or by diazomethane gave this ester which underwent further methylation by the Purdie method faster than the original acid or ester, and very rapidly by a modified Purdie reaction in *NN*-dimethylformamide. Picrotoxic acid was similarly converted into methyl dihydrodi-*O*-methylpicrotoxate by way of methyl *O*-methylpicrotoxate (identical with Sutter and Schlittler's " substance C " [3]) and methyl di-*O*-methylpicrotoxate. Dihydropicrotoxic acid was recovered on demethylation of methyl dihydro-*O*-methylpicrotoxate and the related dimethyl ether by acid. Hydrolysis of methyl dihydropicrotoxate or its methyl ether by alkali gave the corresponding acids. Methyl dihydrodi-*O*-methyl picrotoxate was resistant to alkali; but treatment with hydrazine hydrate in boiling 2-ethoxyethanol gave the acid, and methylamine was liberated ($R\cdot CO_2Me + N_2H_4,H_2O \longrightarrow R\cdot CO_2H + NH_2\cdot OH + NH_2Me$). Methylation of dihydro-*O*-methylpicrotoxic and dihydrodi-*O*-methylpicrotoxic acid with diazomethane regenerated the esters. Infrared evidence confirms the assignment of functional groups involved in the reactions between these compounds. Thus methyl dihydrodi-*O*-methylpicrotoxate shows no bands due to hydroxyl, but bands at 1100—1120 cm.$^{-1}$ due to ether linkages, at 1734 cm.$^{-1}$ due to an ester-carboxyl group, and at 1785 cm.$^{-1}$ due to an unreactive γ-lactone group.

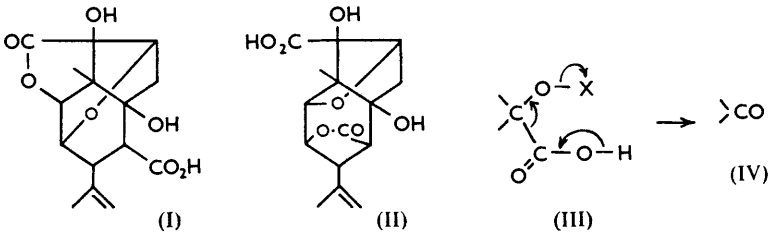
Dihydropicrotoxic acid is stable to dichromate in dilute sulphuric acid at 100°, fuming nitric at 100°, boiling 2N-potassium permanganate, Oppenauer reagents, red phosphorus and boiling hydriodic acid, and glycol-splitting reagents. The hydroxyl groups of picrotoxic and dihydropicrotoxic acid are therefore tertiary and not attached to adjacent carbon atoms, and suppression of the double bond by hydrogenation arrests aromatisation. One of the hydroxyl groups of these acids is acidic, as it is methylated by diazomethane and the esters are soluble in alkali. That the acidity [4] of methyl picrotoxate is due to the hydroxyl group rather than to lactone fission is shown by the alkali-insolubility of methyl *O*-methylpicrotoxate. This acidic hydroxyl group therefore belongs to an α-hydroxycarboxylic group, and in this connection the similar acidic properties of methyl quinate

[1] Conroy, *J. Amer. Chem. Soc.*, 1952, **74**, 491.
[2] Mercer, Robertson, and Cahn, *J.*, 1935, 997; Mercer and Robertson, *J.*, 1936, 288; Harland and Robertson, *J.*, 1939, 935; Sutter and Schlittler, *Helv. Chim. Acta*, 1947, **30**, 403, 2102.
[3] Sutter and Schlittler, *Helv. Chim. Acta*, 1950, **33**, 902.
[4] Horrmann, *Annalen*, 1916, **411**, 273.

may be cited. Since this carboxyl group belongs either to the free carboxylic acid group or to the γ-lactone group, two formulæ are possible for picrotoxic acid (I, II) on the assumption of Conroy's carbon skeleton for picrotoxinin.[1] I am much indebted to a Referee for pointing out that the dihydro-derivative of an acid (II) should be cleaved by oxidising agents such as nitric acid in the sense (III)—(IV), and that absence of such fission and of reaction with lead tetra-acetate, points to formula (I) for picrotoxic acid. I agree with this view.

O-Dihydrodimethylnorpicrotoxamine and the corresponding amine from dihydropicrotoxic acid were isolated in yields unpromising for further degradation.

The bands at 3610 and 3500 cm.$^{-1}$ in the spectrum of picrotoxinin are due to a hydroxyl group, previously suspected[4] from Zerewitinoff estimation. This hydroxyl group is tertiary as picrotoxinin cannot be oxidised to a ketone or acetylated by the usual pro-



(I) (II) (III) (IV)

cedure. Picrotoxinin has now been acetylated by boiling acetic anhydride and sodium acetate: the monoacetate obtained could not be prepared from Horrmann's anhydropicrotin.[5] Picrotoxinin acetate gave picrotoxic acid on acid hydrolysis, α-dihydropicrotoxinin acetate on hydrogenation, methyl *O*-methylpicrotoxate on treatment with diazomethane and potassium hydroxide, and a 6 : 1 mixture of α- and β-picrotoxinone acetate on oxidation by chromic oxide or ozonolysis. Ozonolysis therefore gave an equilibrium mixture of keto–enol isomers, for the α-isomer changed into the β-isomer when heated, both isomers gave a single crystalline 2 : 4-dinitrophenylhydrazone, and the α-isomer was not reducible catalytically. The acetylation product of picrotoxinin is therefore a normal array of functional groups, $C_{11}H_{10}O_3(\cdot CO\cdot O\cdot)(OAc)(CMe{:}CH_2)$. In contrast to the other picrotoxinin derivatives, it has the convulsant properties of the parent compound, and this can be interpreted as biological evidence of similar chemical structure. Its infrared spectrum is similar to that of the parent compound—bands at 1645 and 896 cm.$^{-1}$ due to the *iso*propenyl group, and at 1125 cm.$^{-1}$ due to an ether linkage. Picrotoxinin shows intense split peaks at 1780 and 1765 cm.$^{-1}$, and the acetate an intense band at 1785 cm.$^{-1}$, all due to two γ-lactone groups. In the latter compound, the split peak is lost on account of the presence at 1730 cm.$^{-1}$ of a band due to an acetate group, a view which is confirmed by the presence of a further band at 1235 cm.$^{-1}$. The hydroxyl band of picrotoxinin has previously been mentioned. The presence of two γ-lactone groups in these compounds can only be deduced from the intensity of the split peaks, and in this respect the earlier deductions by Conroy[1] and by Benstead *et al.*[6] require modification. Santonin has now been used for comparison. Intense split peaks at 1795 and 1772 cm.$^{-1}$ for α-dihydropicrotoxinin, and at 1805 and 1774 cm.$^{-1}$ for picrotin agree with the presence of two γ-lactone groups.

Treatment of picrotoxinin with hydroxylamine did not effect lactone fission,[7] and in order to achieve a stepwise degradation procedure reduction of picrotoxinin and picrotin

[5] Horrmann, *Ber.*, 1910, **43**, 1903.
[6] Benstead, Brewerton, Fletcher, Martin-Smith, Slater, and Wilson, *J.*, 1952, 1042.
[7] Clemo and Cocker, *J.*, 1946, 30.

by lithium aluminium hydride and treatment of the resulting polyhydric alcohols with glycol-splitting reagents was initiated.[8]

Experimental

Methyl Dihydro-O-methylpicrotoxate.—(*a*) An acetone solution (200 ml.) of anhydrous dihydropicrotoxic acid [2] (5 g.) was refluxed with methyl sulphate (4 ml.) and potassium carbonate (10 g.) for 5 hr. Crude *methyl dihydro-O-methylpicrotoxate*, recovered in the usual way, crystallised from water in stout needles (4·6 g.), m. p. 165°, $[\alpha]_D^{20}$ +94° (*c* 1·0 in EtOH) [Found: C, 60·0; H, 7·1; OMe, 18·2. $C_{15}H_{18}O_5(OMe)_2$ requires C, 60·0; H, 7·10; OMe, 18·2%], insoluble in aqueous alkali.

(*b*) A suspension of anhydrous dihydropicrotoxic acid (1 g.) in absolute methanol (20 ml.) was treated with excess of diazomethane. The product (0·9 g.) crystallised from water, forming needles, m. p. 165°, alone or mixed with methyl dihydro-*O*-methylpicrotoxate.

Methyl Dihydrodi-O-methylpicrotoxate.—(*a*) A methyl iodide solution (25 ml.) of methyl dihydro-*O*-methylpicrotoxate (2 g.) was refluxed for 2 days with freshly prepared silver oxide [9] (4 g.) and anhydrous sodium sulphate (1 g.), 4 further additions of silver oxide (1 g. each) being made at 10 hr. intervals. Methyl dihydrodi-*O*-methylpicrotoxate (2·03 g.) crystallised from light petroleum (b. p. 80—100°) in stout needles, m. p. and mixed m. p. 146—147°, $[\alpha]_D^{20}$ +112° (*c* 1·0 in EtOH) [Found: C, 60·9; H, 7·4; OMe, 26·1. Calc. for $C_{15}H_{17}O_4(OMe)_3$: C, 61·05; H, 7·3; OMe, 26·2%].

(*b*) Methyl dihydro-*O*-methylpicrotoxate (10 g.) in *NN*-dimethylformamide (5 ml.) was refluxed with methyl iodide (10 ml.), silver oxide (10 g.), and anhydrous sodium sulphate (1 g.) for 3 hr. Methyl dihydrodi-*O*-methylpicrotoxate was precipitated from dimethylformamide solution by water, and crystallised from light petroleum, forming needles, m. p. 146—147°. This ester is vesicant and is stable to boiling aqueous 50% potassium hydroxide.

Methyl O-Methylpicrotoxate.—(*a*) An acetone solution (100 ml.) of methyl picrotoxate [4] (3·25 g.) was similarly refluxed with methyl sulphate (2·2 ml.) and potassium carbonate (5 g.) for 5 hr. Methyl *O*-methylpicrotoxate crystallised from water in prisms (2·6 g.), m. p. 177° (depressed to *ca.* 150° by admixture with methyl picrotoxate, m. p. 172°, and undepressed by admixture with Sutter and Schlittler's [3] " substance C "), $[\alpha]_D^{20}$ +105° (*c* 2·2 in EtOH) (Found: C, 60·6; H, 6·4; OMe, 17·4. Calc. for $C_{17}H_{22}O_7$: C, 60·4; H, 6·5; OMe, 18·3%). Unlike methyl picrotoxate,[4] methyl *O*-methylpicrotoxate was insoluble in aqueous alkali.

(*b*) Methyl picrotoxate (1 g.) in methanol (10 ml.) was methylated by excess of diazomethane, and methyl *O*-methylpicrotoxate (1 g.) was obtained in prisms, m. p. 177°, undepressed by Sutter and Schlittler's [3] " substance C ".

Methyl Di-O-methylpicrotoxate.—Methyl *O*-methylpicrotoxate (2·4 g.) in *NN*-dimethylformamide (4 ml.) was refluxed with methyl iodide (10 ml.), silver oxide (4 g.), and anhydrous sodium sulphate (1 g.) for 3 hr. Dilution of the dimethylformamide filtrate precipitated *methyl di-O-methylpicrotoxate* (2·3 g.) which crystallised from light petroleum (b. p. 100—120°) in lamellæ (2 g.), m. p. 124°, $[\alpha]_D^{20}$ +124° (*c* 2·1 in EtOH) (Found: C, 61·4; H, 6·8; OMe, 26·4. $C_{18}H_{24}O_7$ requires C, 61·4; H, 6·8; OMe, 26·4%). The same products were also obtained from picrotoxic acid.[4]

Hydrogenation of Methyl Di-O-methylpicrotoxate.—An ethanolic solution of this ester (1·75 g.) was hydrogenated at 1 atm., in the presence of Adams platinic oxide catalyst and one drop of 12N-hydrochloric acid. Methyl dihydrodi-*O*-methylpicrotoxate (1·7 g.) crystallised from light petroleum (b. p. 80—100°) in needles, m. p. and mixed m. p. 146—147°.

Demethylation of Methyl Dihydro-O-methylpicrotoxate.—Methyl dihydro-*O*-methylpicrotoxate (3 g.) was refluxed with 90% orthophosphoric acid (40 ml.) for 1 hr., then the mixture was diluted and extracted with ether for 1 day. Evaporation left the crude product which was dissolved in 2N-sodium hydrogen carbonate, and the whole then extracted with ether for 1 day. Acidification of the aqueous phase with hydrochloric acid gave dihydropicrotoxic acid (2·6 g.), m. p. 260°, isolated by extraction and crystallisation from ethyl acetate.

Demethylation of Methyl Dihydrodi-O-methylpicrotoxate.—Methyl dihydrodi-*O*-methylpicrotoxate (2 g.) was refluxed with freshly distilled hydriodic acid (25 ml., *d* 1·7) and 90%

[8] Holker, Holker, McGookin, Robertson, Sergeant, and Hathway, *J.*, 1957, 3746.
[9] Helferich and Klein, *Annalen*, 1926, **450**, 219.

orthophosphoric acid (10 ml.) for 4 hr., then the mixture was diluted and worked up as in the preceding experiment. Dihydropicrotoxic acid (1·8 g.) crystallised from ethyl acetate, forming needles, m. p. and mixed m. p. 260°.

Hydrolysis of Methyl Dihydro-O-methylpicrotoxate.—Methyl dihydro-*O*-methylpicrotoxate (2 g.) was refluxed with 2N-potassium hydroxide (25 ml.) for 2 hr. Acidification yielded felted needles of *dihydro-O-methylpicrotoxic acid* which crystallised from water in parallelepipeds, m. p. 240°, $[\alpha]_D^{20}$ +86° (*c* 1·1 in EtOH) (Found: C, 58·85; H, 6·5. $C_{16}H_{22}O_7$ requires C, 58·9; H, 6·75%). Treatment of a solution of the acid (500 mg.) in ether with excess of diazomethane regenerated the ester (500 mg.), m. p. and mixed m. p. 165°.

Hydrolysis of Methyl Dihydrodi-O-methylpicrotoxate.—A 2-ethoxyethanol solution (27 ml.) of methyl dihydro-*O*-dimethylpicrotoxate (5 g.) was refluxed with 60% hydrazine hydrate (50 ml.) for 30 hr., nitrogen being passed successively through the mixture and 0·1N-hydrochloric acid (15 ml.). The reaction mixture was diluted, acidified with 12N-hydrochloric acid, then kept at 0°. Dihydrodi-*O*-methylpicrotoxic acid was recovered and crystallised from water in needles (4·95 g.), m. p. 206—207°, $[\alpha]_D^{20}$ +109° (*c* 1·1 in EtOH) [Found: OMe, 18·2. Calc. for $C_{15}H_{18}O_5(OMe)_2$: OMe, 17·9%]. Concentrated aqueous hexachloroplatinic acid (5 ml.) was added to the contents of the gas absorption vessel which was then kept at 0°. Methylamine chloroplatinate (3 g.) was recovered and dried at 100°/15 mm. [Found: C, 5·0; H, 2·0; N, 6·0. $(CH_5N)_2PtCl_6$ requires C 5·1; H 2·1; N, 6·0%]. Treatment of an ether solution of dihydrodi-*O*-methylpicrotoxic acid (500 mg.) with excess of diazomethane regenerated methyl dihydrodi-*O*-methylpicrotoxate (500 mg.), m. p. and mixed m. p. 146—147°.

Attempted Oxidations of Dihydropicrotoxic Acid.—This acid was recovered unchanged from treatment with: (1) N-sodium dichromate in 2N-sulphuric acid at 100° for 8 hr., (2) nitric acid (*d* 1·45) at 100° for 5 hr., (3) excess of boiling 2N-potassium permanganate for 6 hr., (4) excess of *cyclo*hexanone and aluminium *tert.*-butoxide in boiling absolute dioxan for 6 hr., (5) red phosphorus and excess of boiling hydriodic acid (*d* 1·7) for 6 hr., (6) excess of lead tetra-acetate in warm acetic acid, and (7) excess of periodic acid in aqueous ethanol.

Schmidt Reaction with Dihydrodi-O-methylpicrotoxic Acid.—To a stirred mixture of a sulphuric acid solution (15 ml.; *d* 1·84) of dihydrodi-*O*-methylpicrotoxic acid (2·3 g.) and chloroform (35 ml.) at 50°, sodium azide (520 mg.) was added portionwise throughout 1 hr., and the mixture was stirred for a further 1 hr. at 50°, then kept at 20° for 1 day, and diluted with ice. The aqueous phase was neutralised with sodium hydrogen carbonate and extracted with ether for 2 days. Evaporation of the solvent left an oily amine (0·5 ml.) which did not liberate ammonia on treatment with boiling alkali. With acetic anhydride–pyridine the product gave N-*acetyldihydrodi-O-methylnorpicrotoxamide* which formed rhombs, m. p. 285°, from dilute acetic acid [Found: C, 61·0; H, 7·55; N, 4·1. $C_{14}H_{17}O_3(OMe)_2 \cdot NHAc$ requires C, 61·2; H, 7·65; N, 4·0%].

Dihydrodi-O-methylpicrotoxamide crystallised from dilute acetic acid in felted needles, m. p. 262° [Found: C, 60·3; H, 7·5; N, 4·2. $C_{14}H_{17}O_3(OMe)_2 \cdot CO \cdot NH_2$ requires C, 60·2; H, 7·4; N, 4·15%], and was converted under the conditions of the Hofmann reaction into the same amine, characterised as *N*-acetyl derivative, m. p. 285°.

Schmidt Reaction with Dihydropicrotoxic Acid.—When a stirred mixture of a sulphuric acid solution (40 ml.; *d* 1·84) of dihydropicrotoxic acid (6·25 g.) and chloroform (80 ml.) at 50° was treated with sodium azide (1·82 g.) as above, extraction of the neutralised aqueous phase yielded a small quantity of amine which on treatment with acetic anhydride–pyridine gave a *triacetate*, needles (from dilute acetic acid) (3 mg.), m. p. 287° (Found: C, 59·2; H, 6·5. $C_{20}H_{27}O_8N$ requires C, 59·0; H, 6·6%) (test for N positive).

Picrotoxinin Acetate.—When picrotoxinin (5 g.) was refluxed with acetic anhydride (15 ml.) and fused sodium acetate (300 mg.) for 16 hr., crude product recovered in the usual way crystallised from acetic acid (charcoal) in stout pyramids (3 g.), m. p. 255°, $[\alpha]_D^{20}$ +18° (*c* 1·7 in C_5H_5N), of *picrotoxinin acetate* (Found: C, 61·1; H, 5·5; Ac, 15·7. $C_{17}H_{18}O_7$ requires C, 61·1; H, 5·4; Ac, 13·0%). Horrmann's anhydropicrotin,[5] m. p. 315° (decomp.) (Found: C, 61·4; H, 5·5. Calc. for $C_{15}H_{16}O_6$: C, 61·6; H, 5·5%), remained unchanged by this treatment.

Hydrogenation of Picrotoxinin Acetate.—Hydrogenation of an acetic acid solution (100 ml.) of picrotoxinin acetate (750 mg.) at 1 atm., in the presence of Adams platinic oxide and 1 drop of 12N-hydrochloric acid gave *α-dihydropicrotoxinin acetate* (700 mg.) which crystallised from acetic acid in hexahedra, m. p. 250° (undepressed by admixture with a specimen prepared by direct acetylation of α-dihydropicrotoxinin), $[\alpha]_D^{20}$ +14° (*c* 1·8 in C_5H_5N) (Found: C, 60·5; H, 6·25. $C_{17}H_{20}O_7$ requires C, 60·7; H, 5·9%).

Acid-hydrolysis of Picrotoxinin Acetate.—Picrotoxinin acetate (350 mg.) was refluxed with N-sulphuric acid (20 ml.) for 1 day, then a small volume of distillate was collected and shown to contain acetic acid by the isolation of 2-methylbenziminazole picrate, m. p. and mixed m. p. 214°. Picrotoxic acid (300 mg.) was recovered as needles, m. p. and mixed m. p. 230°.

Reaction of Picrotoxinin Acetate with Diazomethane.—A methanolic solution (20 ml.) of picrotoxinin acetate (1 g.) was treated with excess of diazomethane and an aqueous solution (0·5 ml.) of potassium hydroxide (50 mg.) for 2 days. Methyl *O*-methylpicrotoxate, isolated in the usual way, crystallised from water in needles (900 mg.), m. p. 176°, identical with Sutter and Schlittler's " substance C." [3]

Ozonolysis of Picrotoxinin Acetate.—Ozonised oxygen was conducted through an ethyl acetate solution (700 ml.) of picrotoxinin acetate (4 g.) at 0° for 5 hr. The ozonide gradually separated in prisms; it was decomposed with ice-water; the crude product (3·8 g.) melted at 189°. A hot ethanolic solution (300 ml.) of the product deposited rhombs (3 g.), m. p. 212—214°, of *α-picrotoxinone acetate*, $[\alpha]_D^{20}$ +89° (*c* 0·6 in C_5H_5N) (Found: C, 57·1; H, 4·9. $C_{16}H_{16}O_8$ requires C, 57·1; H, 4·8%). Partial evaporation of the mother-liquors gave crystals which recrystallised from acetic acid in cubes (0·5 g.), m. p. 276° (decomp.), of *β-picrotoxinone acetate*, $[\alpha]_D^{20}$ +80° (*c* 0·2 in C_5H_5N) (Found: C, 57·0; H, 4·7%), identical with the product obtained from the α-isomer at 220°/0·01 mm. Treatment of an aqueous solution of α- or β-picrotoxinone acetate (100 mg.) with 2 : 4-dinitrophenylhydrazone sulphate reagent in 10N-sulphuric acid gave a precipitate of 2 : 4-*dinitrophenylhydrazone* which crystallised from chloroform–alcohol in orange cubes, m. p. 256° (decomp.) (Found: C, 51·1; H, 3·7; N, 11·0. $C_{22}H_{20}O_{11}N_4$ requires C, 51·2; H, 3·9; N, 10·9%). In a parallel experiment, the distillate from the decomposition of the ozonide was used for the estimation and identification of formaldehyde (0·5 mol.) as the dimedone derivative, m. p. 189—191°. An acetic acid solution of α-picrotoxinone acetate was recovered unchanged after attempted hydrogenation at 1 atm. in the presence of Adams platinic oxide.

Oxidation of picrotoxinin acetate with chromium trioxide in acetic acid similarly afforded a mixture (6 : 1) of α- and β-picrotoxinone acetate.

Attempted Reaction of Picrotoxinin with Hydroxylamine.—Picrotoxinin was recovered unchanged from treatment of a boiling ethanolic solution (1 g. in 6 ml.) with hydroxylamine hydrochloride (1 g.) and fused sodium acetate (2 g.) for 5 hr.

Silver Oxide Oxidation of Picrotoxinin.—An aqueous solution (200 ml.) of picrotoxinin (2·9 g.) was refluxed with silver oxide (4 g.) for 2 hr., then the filtrate was acidified with 12N-hydrochloric acid, silver halide removed, and the filtrate neutralised with sodium hydrogen carbonate. Extraction with ether and evaporation gave a residue of picrotoxinin which crystallised from water in needles (50 mg.), m. p. 204—205°. Extraction of the re-acidified aqueous phase with ether gave a residue (1 g.) which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms, m. p. 290°, of αα-dimethylphthalide-3 : 4-dicarboxylic acid [10] (Found: C, 54·0; H, 4·7. Calc. for $C_{12}H_{10}O_6,H_2O$: C, 53·75; H, 4·5%).

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[10] Hansen, *Ber.*, 1933, **66**, 849.