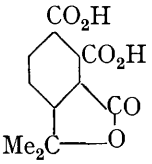
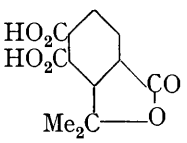
230. *Picrotoxin. Part I. The Constitution of Picrotic Acid and the C-Skeleton of Picrotoxinin and Picrotin.*

By DONALD MERCER, ALEXANDER ROBERTSON, and (in part) ROBERT S. CAHN.

BY the action of boiling hydriodic acid and phosphorus on the two dilactones, picrotoxinin, $C_{15}H_{16}O_6$, and picrotin, $C_{15}H_{18}O_7$, which constitute the molecular compound picrotoxin (Bakunin and Giordani, *Rend. Accad. Sci. Fis. Mat. Napoli*, 1924, iii, **30**, 166, who give references to the earlier views on the nature of the complex) Angelico (*Gazzetta*, 1911, **41**, ii, 337) obtained the same products, picrotic acid, $C_{15}H_{18}O_4$ (Oglialoro, *Gazzetta*, 1891, **21**, ii, 213), and a ketone, $C_{14}H_{16}O_3$, thus showing that in all probability the two lactones possessed the same *C*-skeleton. This conclusion is supported by the formation of the same chloro-ketone, $C_{14}H_{15}O_3Cl$, from both lactones with hydrochloric acid at 180°. On oxidising picrotic acid, Angelico (*loc. cit.*) obtained a dibasic acid, $C_{12}H_{12}O_7$, m. p. 289°, apparently identical with the acid, $C_{12}H_{10}O_6,H_2O$, which this author subsequently obtained from the ketone $C_{14}H_{16}O_3$ (*Gazzetta*, 1912, **42**, ii, 540) and to which he ascribed the structure (I).



(I.)



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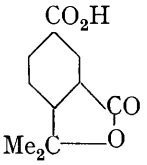


(III.)

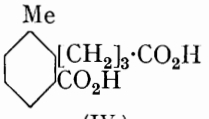
By the oxidation of picrotoxinin and of picrotin with manganese dioxide and hot sulphuric acid Hansen (*Ber.*, 1933, **66**, 850) obtained a phthalic acid, $C_{12}H_{10}O_6$, m. p. 220° (anhydride, m. p. 286°), which he observed had the properties of Angelico's acid and which he was able to degrade by way of the imide to a monobasic acid, $C_{11}H_{10}O_4$, m. p. 202°. Further, on decarboxylation the latter acid yielded αα-dimethylphthalide. Though Hansen did not orient the acids $C_{11}H_{10}O_4$ and $C_{12}H_{10}O_6$, he concluded that, since the latter compound was accompanied by benzene-1 : 2 : 3 : 4-tetracarboxylic acid in the oxidation mixture, it had either formula (I) or (II), of which he preferred (I) in agreement with the conclusions of Angelico (*loc. cit.*).

It was noted by one of us (R. S. C.) that the properties of Hansen's acid $C_{11}H_{10}O_4$ were identical with those of cannabinolactonic acid (III) (J., 1932, 1352), and this view has been confirmed by direct comparison of the two compounds and of their ethyl esters. The acid $C_{12}H_{10}O_6$ has been oxidised with nitric acid to benzene-1 : 2 : 3 : 4-tetracarboxylic acid and hence must have the structure (I). Further, this acid, $C_{12}H_{10}O_6$, has also been obtained by the oxidation of picrotic acid, thereby confirming the identity of Angelico's and Hansen's products and affording direct evidence of the presence of an αα-dimethylphthalide group in picrotic acid.

As the result of degradation experiments Hormann (*Ber.*, 1916, **49**, 2107) showed that picrotic acid contained a side chain $\cdot C_3H_6\cdot CO_2H$, but he did not determine the position of the carboxyl group in this residue. Hydrolytic fission of picrotic acid was found by Angelico and Monforte (*Gazzetta*, 1923, **53**, 800) to yield acetone and a dibasic acid, $C_{12}H_{14}O_4$, which was considered by them to retain the side chain $\cdot C_3H_6\cdot CO_2H$. For this product these authors suggested two possible formulæ, (IV) and (V), of which they preferred the latter (V), and hence they ascribed formula (VI) to picrotic acid, basing their views on (*a*) the presence of an αα-dimethylphthalide group indicated by fission to acetone and the acid $C_{12}H_{14}O_4$ with the formation of a new carboxyl group, (*b*) the assumption that the residue $\cdot C_3H_6\cdot CO_2H$ was a straight chain, and (*c*) the fluorescein reaction which they claim to have obtained with an unidentified fusion product of an acid, $C_{15}H_{16}O_6$, resulting from the oxidation of picrotic acid.

By analogy with the related lactones santonin (Clemo and co-workers, J., 1929, 2368), artemisin, alantolactone, and *iso*alantolactone (*Ann. Reports*, 1932, 158) it was considered highly probable that picrotin and picrotoxinin were related to the sesquiterpene group.

The *C*-skeleton implied for the picrotoxin lactones by formula (VI) for picrotic acid is difficult to reconcile with this hypothesis, but if picrotic acid is formulated as (VII),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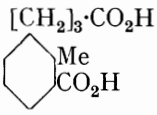
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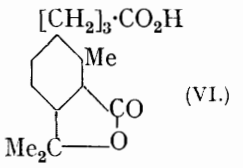
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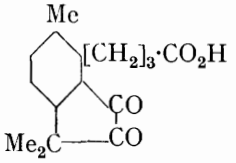
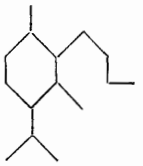
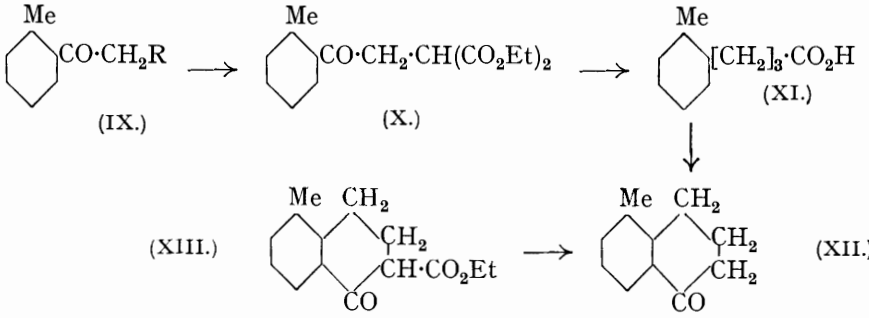
(VII.)




(VIII.)

implying, as we believe it does, the same *C*-skeleton (VIII) for the lactones, then their relationship to the cadinene group is at once apparent. On these grounds it appeared that the possibility of picrotic acid having formula (VI) could be neglected and we were led directly to deduce the structure (VII) for this compound as follows :

Application of the Dieckmann reaction to the ethyl ester of the acid, $C_{12}H_{14}O_4$, obtained by hydrolytic fission of picrotic acid gave rise to the β-ketonic ester (XIII), the structure of which is established by hydrolysis to the tetralone (XII), identical with an authentic specimen. It is clear, therefore, that the dibasic acid $C_{12}H_{14}O_4$ which can give rise to (XIII) must have formula (IV) and since this acid is obtained from picrotic acid, which has been shown to embody an αα-dimethylphthalide group, the latter acid must have the structure (VII) and not (VI).


(IX.) (X.) (XI.) (XIII.) (XII.)

The authentic 5-methyl-α-tetralone mentioned above has been synthesised by a new method (compare Heilbron and co-workers, J., 1930, 425). The condensation of (IX, R = Br) with ethyl sodiomalonate gave the keto-ester (X), which on reduction and subsequent hydrolysis and decarboxylation of the product gave rise to (XI). The orientation of (X) and also that of (IX, R = Br) was confirmed by the production of the keto-ester (X) from (IX, R = Cl) obtained from *o*-toluoyl chloride by Robinson and Bradley's method (J., 1928, 2904; 1930, 793). Cyclisation of the acid (XI) with sulphuric acid yielded the tetralone (XII) in a yield superior to that obtained by the action of aluminium chloride on the acid chloride.

Experimental.

αα-Dimethylphthalide-4-carboxylic Acid (*Cannabinolactonic Acid*) (III).—When the quantity of pure manganese dioxide indicated by Hansen (*loc. cit.*) for the preparation of αα-dimethylphthalide-3 : 4-dicarboxylic acid was used, only traces of this acid were obtained. The following procedure gave satisfactory results : To a stirred solution of picrotoxin (8 g.) in boiling 57% sulphuric acid (300 c.c.), manganese dioxide (70 g.) was added during 5—6 hours, and the mixture then refluxed for 16—18 hours. On isolation from the filtered solution by ten extractions with ether the acid, m. p. 215—220° (anhydride crude, m. p. 280—284°; pure, m. p. 290°), was converted into the imide and thence into 3-amino-αα-dimethylphthalide-4-carboxylic acid by

Hansen's method (Angelico, *loc. cit.*, and Hansen, *loc. cit.*, respectively give m. p. of anhydride as 289° and 286°). The latter product * melted 183—200° and could not be readily purified by crystallisation, but elimination of the amino-group gave rise to cannabinolactonic acid, which separated from benzene in colourless prisms, m. p. 202—203°, and was identical with a specimen obtained from cannabinol and with a synthetical specimen prepared by Bargellini and Forli-Forti's method (*Gazzetta*, 1910, **40**, ii, 74) (Found : C, 64·2; H, 4·9. Calc. for $C_{11}H_{10}O_4$: C, 64·0; H, 4·9%). The ethyl ester separated from alcohol in stout needles and was identical with an authentic specimen (Bargellini and Forli-Forti, *Gazzetta*, *loc. cit.*), m. p. 104° (Found : C, 66·9; H, 6·1. Calc. for $C_{13}H_{14}O_4$: C, 66·7; H, 6·0%).

Oxidation of αα-Dimethylphthalide-3 : 4-dicarboxylic Acid.—The acid (1 g.) was heated with 33% nitric acid (20 c.c.) in a sealed tube at 170° for 24 hours, the mixture diluted with water, and the nitric acid removed by distillation with the occasional addition of water to maintain the volume at 40—80 c.c.; the resulting solution was neutralised with ammonia, and benzene-1 : 2 : 3 : 4-tetracarboxylic acid isolated as the lead salt. A boiling aqueous solution of the acid obtained by decomposition of the lead salt with hydrogen sulphide was treated with charcoal, filtered, and allowed to evaporate at room temperature in a vacuum. On esterification with ethereal diazomethane the crude crystalline acid (0·25 g.), m. p. 228—234°, gave the tetramethyl ester, m. p. 132° after purification, undepressed by admixture with the ester obtained from an authentic specimen of the acid (Freund and Fleischer, *Annalen*, 1916, **411**, 14) (Found : C, 54·0; H, 4·4. Calc. for $C_{14}H_{14}O_8$: C, 54·2; H, 4·6%).

Oxidation of Picrotic Acid.—Manganese dioxide (45 g.) was added to a boiling solution of picrotic acid (Angelico, *loc. cit.*) (8 g.) in 57% sulphuric acid (220 c.c.) in the course of 2 hours, the mixture refluxed for 15 hours, diluted with water (450 c.c.), and filtered, and the product isolated by eight extractions with ether. One half of the crude material was refluxed with acetyl chloride (7 c.c.) for ½ hour, and the resulting solid recrystallised from acetic anhydride, forming colourless plates (1·5 g.), m. p. 290°, identical with an authentic specimen of the anhydride of αα-dimethylphthalide-3 : 4-dicarboxylic acid obtained from picrotoxin.

Ethyl 5-Methyl-α-tetralone-2-carboxylate (XIII).—6-Carboxy-γ-*o*-tolyl-*n*-butyric acid (IV), m. p. 135—136°, was prepared from picrotoxin *via* picrotic acid according to the directions of Angelico (*loc. cit.*), who gives m. p. 132°; the ethyl ester was a colourless oil, b. p. 158—160°/1 mm.

This ester (7 g.) was gradually added in the course of 1 hour to powdered sodium (1·2 g.) in toluene (45 c.c.) on the steam-bath, and the reaction completed by heating for a further 4 hours. Next day an excess of dry ether was added, the bulky precipitate of the sodium derivative of ethyl 5-methyl-α-tetralone-2-carboxylate separated and decomposed with dilute acetic acid, and the keto-ester isolated with ether. Purified by distillation in a vacuum, the compound was obtained as a colourless oil (2·8 g.), b. p. 152—155°/1 mm., which gave a blue coloration with alcoholic ferric chloride. On being refluxed with phenylhydrazine (1·2 mols.) in alcohol containing a little acetic acid for 70 minutes, this compound formed a *pyrazolone*, which crystallised from alcohol in colourless prisms, m. p. 265° (Found : N, 10·2. $C_{18}H_{16}ON_2$ requires N, 10·1%).

5-Methyl-α-tetralone (XII).—The foregoing keto-ester (1·9 g.) was refluxed with 20% sulphuric acid (10 c.c.) for 1 hour, and the product distilled with steam, isolated with ether, and converted into the semicarbazone (1·5 g.). Hydrolysis of the crude solid with boiling 10% hydrochloric acid (10 c.c.) for 1 hour, followed by steam-distillation, gave almost pure tetralone, which formed large prisms from light petroleum (b. p. 40—60°), m. p. 49—50°, undepressed by admixture with authentic material. The *semicarbazone* separated from alcohol in needles, m. p. 245—246° (Found : N, 19·4. $C_{12}H_{15}ON_3$ requires N, 19·4%). Prepared by Brady's method (J., 1931, 756), the 2 : 4-*dinitrophenylhydrazone* crystallised from ethyl acetate in slender red needles, m. p. 229—230° (Found : C, 60·4; H, 4·7; N, 16·5. $C_{17}H_{16}O_4N_4$ requires C, 60·0; H, 4·7; N, 16·4%). The phenylhydrazone separated from alcohol in plates, m. p. 105°, but decomposed on being kept. These derivatives were identical with the respective authentic specimens obtained from the synthetic tetralone.

o-*Methylacetophenone.*—A solution of *o*-toluoyl chloride (Klages, *Ber.*, 1899, **32**, 1549) (50 g.) in absolute ether (50 c.c.) was gradually added to ethyl sodioacetoacetate (from 50 g. of the

* In consideration of the work of Rodinow and co-workers (*Ber.*, 1929, **62**, 2563), who by a similar procedure obtained the two theoretically possible anthranilic acids from hemipinimide, degradation of the above imide would be expected to give a mixture of two aminocannabinolactonic acids. This would account for the contamination of 3-aminocannabinolactonic acid as indicated by the indefinite m. p. and also for the yield (*ca.* 50% of the theoretical).

ester and 8 g. of sodium) in ether (200 c.c.), and the mixture refluxed for 4 hours to complete the reaction. Enough water was added to dissolve the precipitated salt, the ethereal layer separated, the aqueous solution extracted several times with ether, and the combined ethereal solutions dried and evaporated. Sodium hydroxide (27·5 g.) in water (75 c.c.) was added to a solution of the viscous straw-coloured product (80—90 g.) in alcohol (200 c.c.). This was followed at intervals of 12 hours by four portions of sodium hydroxide (12 g.) in water (75 c.c.) and 12 hours after the last addition the solution was diluted to 1 l. with water and refluxed for 15 hours. *o*-Methylacetophenone (17—18 g.) was isolated with ether and purified by distillation, b. p. 93—95°/15 mm. The semicarbazone had m. p. 210° (Found : N, 22·1. Calc. for $C_{10}H_{13}ON_3$: N, 22·0%) (compare Senderens, *Bull. Soc. chim.*, 1911, **9**, 949, and Auwers, *Annalen*, 1915, **408**, 242, who record m. p. 192° and m. p. 203° respectively).

Unchanged *o*-toluic acid was recovered from the aqueous liquor.

ω-Bromo-o-methylacetophenone (IX, R = Br).—This ketone was prepared from *o*-methylacetophenone (17 g.) in acetic acid (22 c.c.) with bromine (21·3 g.) according to the method used by Rather and Reid (*J. Amer. Chem. Soc.*, 1919, **41**, 77) for the preparation of ω-bromoacetophenone and obtained as a colourless, mobile, lachrymatory oil, b. p. 138—140°/16 mm., which became pale green when kept. The compound (2 g.) was readily characterised by conversion into the corresponding ω-acetoxy-ketone (oil) with sodium acetate (2 g.) and a crystal of potassium iodide in boiling alcohol (17 c.c.) during 15 hours, the *semicarbazone* of which crystallised from alcohol in needles, m. p. 174° (Found : N, 16·8. $C_{12}H_{15}O_3N_3$ requires N, 16·8%).

Ethyl β-o-*Toluoylethane-αα-dicarboxylate* (X).—(A) The afore-mentioned ω-bromo-ketone (44 g.) was carefully added to a solution of ethyl sodiomalonate (from 47 g. of ethyl malonate and 5 g. of sodium) in alcohol (100 c.c.) at 0°. The mixture was kept for 1 day at room temperature, refluxed for ½ hour, diluted with water, and extracted with ether. Evaporation of the dried extract left the keto-ester as a pale yellow oil (25 g.), which was purified by distillation in a high vacuum, b. p. 170°/1 mm. The 2 : 4-*dinitrophenylhydrazone* separated from alcohol in yellow plates, m. p. 136—137° (Found : C, 55·9; H, 5·2; N, 12·1. $C_{22}H_{24}O_8N_4$ requires C, 55·9; H, 5·1; N, 11·9%).

(B) *o*-Toluoyl chloride (10 g.) in ether (70 c.c.) was added to diazomethane (from 34 c.c. of nitrosomethylurethane) in ether (800 c.c.) at − 12° in the course of 15 minutes. Next day the removal of the excess of diazomethane and the solvent left the diazene as an oily residue, which was directly converted into ω-chloro-*o*-methylacetophenone by treatment in ether (75 c.c.) with a slight excess of hydrogen chloride. The strongly lachrymatory liquid was condensed with ethyl sodiomalonate (from 16 g. of the ester and 1·5 g. of sodium) in alcohol (50 c.c.), and the resulting keto-acid (5 g.), (b. p. 165—170°/1 mm.), isolated by the method adopted in the case of the corresponding ω-bromo-ketone.

γ-o-Tolyl-n-butyric Acid (XI).—The keto-ester (10 g.) was reduced by being boiled with amalgamated zinc (65 g.) in alcohol (80 c.c.) and concentrated hydrochloric acid (100 c.c.) for 8 hours; more hydrochloric acid (10 c.c.) was added every 2 hours. The product (7 g.) was isolated with ether and hydrolysed by boiling with potassium hydroxide (20 g.) in 30% alcohol (100 c.c.) for 10 hours. On isolation the resulting dicarboxylic acid was heated at 140° until effervescence ceased (40—50 minutes), a solution of the crude *γ-o*-tolyl-*n*-butyric acid in aqueous sodium bicarbonate treated with charcoal to remove oily insoluble impurities, the filtered solution acidified with hydrochloric acid, and the acid (4 g.) crystallised from light petroleum (b. p. 40—60°), forming colourless prisms, m. p. 60—61° (Heilbron and co-workers, *loc. cit.*, give m. p. 60°), b. p. 140°/1 mm. (Found : C, 74·2; H, 7·8. Calc. for $C_{11}H_{14}O_2$: C, 74·1; H, 7·9%).

Cyclisation of the tolyl-*n*-butyric acid (1 g.) by heating with 95% sulphuric acid (5 c.c.) on the steam-bath for 1 hour gave the tetralone (0·6 g., precipitated with ice-water), which on purification by distillation and then by crystallisation from light petroleum (b. p. 40—60°) formed colourless prisms, m. p. 50—51°, b. p. 116—117°/1 mm., and gave a 2 : 4-dinitrophenylhydrazone, m. p. 229—230° (Found : C, 60·3; H, 4·7; N, 16·3%), a semicarbazone, m. p. 245—246° (decomp.) (Found : N, 19·6%), and an unstable derivative with phenylhydrazine, m. p. 105°.

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