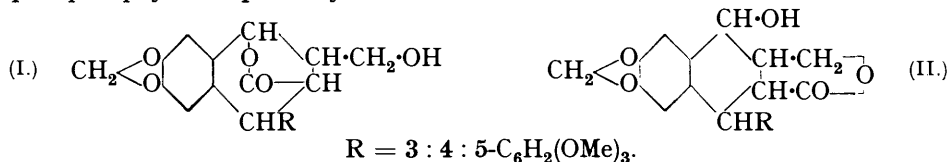


## 28. Podophyllotoxin.

By ALEXANDER ROBERTSON and ROY B. WATERS.

THE results of the earlier investigations on podophyllotoxin, the main constituent of the drug *podophyllin*, though to some extent contradictory, showed that the compound was a levorotatory hydroxy-lactone containing methoxyl groups and that it could be readily converted into the isomeride, picropodophyllin (Podwyssotzki, *Arch. Path. Pharm.*, 1891, **13**, 29; Kürsten, *Arch. Pharm.*, 1891, **229**, 220; Dunstan and Henry, J., 1898, **73**, 212). Borsche and Niemann (*Annalen*, 1932, **494**, 126; **499**, 59) and Späth, Wessely, and Kornfeld (*Ber.*, 1932, **65**, 1536, 1773) have established the empirical formula  $C_{22}H_{22}O_8$  and have isolated a number of degradation products, the most important of which are podophyllomerol and podophyllomeronic, trimethylgallic,\* hydrastic, pyromellitic, and benzenepentacarboxylic acids. In consequence they propose formulæ (I) and (II) for podophyllotoxin and picropodophyllin respectively.



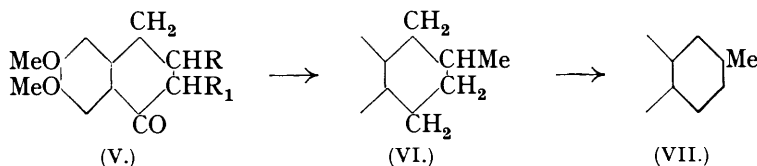
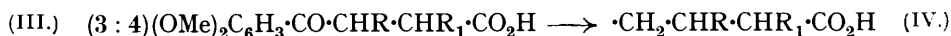
An analytical investigation of podophyllotoxin initiated in 1929, the experimental results of which have already been contained in the memoirs of Borsche and Niemann and of Späth

\* The isolation of trimethylgallic acid as an oxidation product of picropodophyllin was first described by one of us (R. B. W.) in a thesis presented in June, 1931, for the degree of Ph.D. of the University of London. At the same time, following a suggestion by Professor R. Robinson, F.R.S., positive evidence of the presence of a methylenedioxy-group was obtained.

and his co-workers, led us to adopt as a working hypothesis the same carbon skeleton and relative positions of the methoxyl and methylenedioxy-groups as that proposed by these authors.

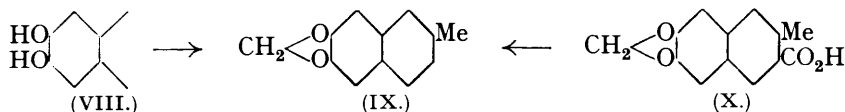
According to Borsche and Niemann the dehydration of picropodophyllin with acetic anhydride and sulphuric acid or with phosphorus pentachloride gives rise to a compound, *apopicropodophyllin*, which in the crude state has m. p. 236—237° (sometimes 15—20° lower). They state that this product is purified by treatment with boiling acetic anhydride and sodium acetate (m. p. 214—216°) and is optically inactive. Späth and his co-workers obtained the compound, m. p. 214—216°, by the action of boiling acetic anhydride on picropodophyllin as well as by the procedure of Borsche and Niemann, and found that it was dextrorotatory. By the dehydration of picropodophyllin, using conditions comparable with those described by Borsche and Niemann, we have obtained a *lævorotatory* lactone, m. p. 244° after purification. This compound, which we have named  $\alpha$ -*apopicropodophyllin*, is readily converted by means of acetic anhydride and sodium acetate (or pyridine) into a dextrorotatory isomeride, m. p. 214—216°,  $\beta$ -*apopicropodophyllin*. Both lactones give rise to the same strongly *lævorotatory* *apopicropodophyllin* acid, but on being heated this acid re-forms  $\alpha$ -*apopicropodophyllin* only.

Podophyllomeronic acid, which was previously prepared from picropodophyllin (*loc. cit.*), has now been obtained from  $\alpha$ - and from  $\beta$ -*apopicropodophyllin*. The constitution ascribed to podophyllomerol, which is formed by the decarboxylation of this acid (*loc. cit.*), has been conclusively established by the following synthesis: Reduction of the mixed keto-acids (III; R = H, R<sub>1</sub> = Me) and (III; R = Me, R<sub>1</sub> = H) resulting from the condensation of veratrole and methylsuccinic anhydride (Fichter and Herbrand, *Ber.*, 1896, 29, 1193) with aluminium chloride gave rise to the acids (IV; R = H, R<sub>1</sub> = Me) and (IV; R = Me, R<sub>1</sub> = H), which on ring closure furnished a mixture of the ketotetrahydronaphthalenes (V; R = H, R<sub>1</sub> = Me) and (V; R = Me, R<sub>1</sub> = H). The latter compounds on reduction gave only one product (VI).



The orientation of the methoxyl groups in ketotetrahydronaphthalenes of the type (V) prepared from veratrole by this route follows from the experiments of Haworth and Mavin (*J.*, 1932, 1485).

The conversion of (VI) into podophyllomerol (IX) was effected through the stages (VII) and (VIII) and the synthetic material was identical with a specimen derived from podophyllomeronic acid.



Thus, since podophyllomeronic acid on oxidation yields pyromellitic acid, it must be represented by formula (X).

Definite experimental evidence regarding the position of the pyrogallol residue and the nature of the free alcoholic hydroxyl group in podophyllotoxin (I) and picropodophyllin (II) is lacking. The ease with which picropodophyllin undergoes dehydration (podophyllotoxin is not dehydrated under the same conditions) and the remarkable extrusion of the pyrogallol residue in the conversion of the tetrahydronaphthalene nucleus of picropodophyllin, etc., into podophyllomeronic acid (X) lead us to the view that podophyllotoxin and



recrystallised from 80% AcOH, forming plates, m. p. 240°, identical with a specimen prepared from picropodophyllin (Found : C, 68.1; H, 4.6. Calc. for  $C_{13}H_{10}O_4$  : C, 67.8; H, 4.4%). It forms in cold conc.  $H_2SO_4$  a pale yellow solution, which on heating becomes red and then purple.

The production of the acid from either source is unaffected by the addition of red P to the reaction mixture.

1- and 4-Keto-6 : 7-dimethoxy-2-methyl-1 : 2 : 3 : 4-tetrahydronaphthalenes.—A mixture of methylsuccinic anhydride (10 g.),  $PhNO_2$  (80 c.c.), veratrole (20 g.), and anhydrous  $AlCl_3$  (38 g.) was kept at room temp. for 50 hr. After the addition of ice (100 g.) and dil. HCl (50 c.c.) the  $PhNO_2$  was removed in steam and the residual oil isolated with  $Et_2O$  and dissolved in aq.  $NaHCO_3$ . The filtered solution (charcoal) was acidified with conc. HCl, and the product obtained as a viscous syrup which did not solidify.

The mixed  $\beta$ -veratrolypropionic acids (22 g.) were reduced with amalgamated Zn (40 g.) and boiling conc. HCl (80 c.c.) during 5 hr., and the product (mixture) isolated with  $Et_2O$  and purified by means of aq.  $NaHCO_3$ .

A mixture of the latter acids and 90%  $H_2SO_4$  (80 c.c.) was heated on the water-bath for 20 min., cooled, and poured on ice (300 g.). A  $CHCl_3$  solution of the semi-solid product was washed with aq.  $NaHCO_3$ , then with dil. aq.  $Na_2CO_3$ , and finally with  $H_2O$ , dried, and evaporated. Distillation of the dark residue in a high vac. gave the mixed ketones as a colourless solid; a specimen cryst. from  $Et_2O$ -light petroleum had m. p. 102—110° (Found : C, 70.6; H, 7.2.  $C_{13}H_{16}O_3$  requires C, 70.9; H, 7.3%).

6 : 7-Dimethoxy-2-methylnaphthalene.—Reduction of the foregoing mixture of ketones (7.5 g.) with amalgamated Zn (25 g.) and warm conc. HCl (40 c.c.) during 5 hr. gave rise to 6 : 7-dimethoxy-2-methyl-1 : 2 : 3 : 4-tetrahydronaphthalene (6.2 g.) as an almost colourless oil, which was dehydrogenated by being heated with powdered Se (12 g.) at 280—290° for 1 hr. and then at 300—310° for 17 hr. A MeOH extract of the reaction mixture was treated with charcoal, filtered, and evaporated. Distillation (twice) of the residue in a high vac. gave a colourless solid (4.5 g.), which had a faint greenish  $FeCl_3$  reaction indicating the presence of a small amount of demethylated product. Addition of an excess of 1% aq. NaOH to a solution of this solid in the minimum amount of MeOH pptd. almost pure 6 : 7-dimethoxy-2-methylnaphthalene, m. p. 95—96°, which crystallised from light petroleum in clusters of elongated prisms, m. p. 100° (Found : C, 77.0; H, 7.0. Calc. for  $C_{13}H_{14}O_2$  : C, 77.2; H, 6.9%) (Borsche and Niemann record m. p. 98—100°).

6 : 7-Dihydroxy-2-methylnaphthalene.—The dimethyl ether (2.5 g.) was heated (oil-bath at 135—140°) with HI (25 c.c.;  $d$  1.7) and  $Ac_2O$  (15 c.c.) for  $\frac{1}{2}$  hr. and, after cooling, a part of the product was pptd. as an almost colourless solid by the addition of an excess of 1% aq.  $NaHSO_3$ ; the remainder was isolated with  $Et_2O$ . Cryst. from  $C_6H_6$ -ligroin, it formed elongated colourless prisms, m. p. 165° (Found : C, 76.0; H, 5.6. Calc. for  $C_{11}H_{10}O_2$  : C, 75.9; H, 5.8%) (Borsche and Niemann give m. p. 161—162°). The substance gives with MeOH- $FeCl_3$  and with EtOH- $FeCl_3$  deep green and pure deep blue colorations respectively; in both cases the colour changes to blue-violet on dilution with  $H_2O$ .

6 : 7-Methylenedioxy-2-methylnaphthalene (Podophyllomerol).—A solution of the foregoing phenol in acetone (20 c.c.) and  $CH_2I_2$  (5 c.c.) was refluxed with anhydrous  $K_2CO_3$  until a sample failed to give a  $FeCl_3$  reaction (5 hr.). More acetone (50 c.c.) was added, the solution filtered, the solvent distilled, and the excess of  $CH_2I_2$  removed in vac. The residue was washed with 1% aq. NaOH and then with  $H_2O$ , dried, and extracted with light petroleum. Evaporation of the filtered extract left podophyllomerol as a colourless solid, which crystallised from a small vol. of MeOH in colourless plates, m. p. 129°, and was identical with a specimen prepared by decarboxylating podophyllomeronic acid (Found : C, 77.2; H, 5.3. Calc. for  $C_{12}H_{10}O_2$  : C, 77.4; H, 5.4%).

The authors are indebted to the Chemical Society for research grants.

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[Received, December 2nd, 1932.]