28. Podophyllotoxin.

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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 lævorotatory 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.

(I.)
$$CH_2 \stackrel{O}{\bigcirc} CH \stackrel{CH \cdot CH_2 \cdot OH}{\bigcirc} CH \stackrel{CH \cdot CH_2 - OH}{\bigcirc} CH \stackrel{CH \cdot CH_2 - O}{\bigcirc} (II.)$$

$$R = 3 : 4 : 5 \cdot C_6 H_2 (OMe)_3.$$

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, apo picropodophyllin, 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 α -apopicropodophyllin, is readily converted by means of acetic anhydride and sodium acetate (or pyridine) into a dextrorotatory isomeride, m. p. 214—216°, β -apopicropodophyllin. Both lactones give rise to the same strongly lævorotatory apopicropodophyllic acid, but on being heated this acid re-forms α -apopicropodophyllin only.

Podophyllomeronic acid, which was previously prepared from picropodophyllin (loc. cit.), has now been obtained from α - and from β -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, $R_1 = Me$) and (III; R = Me, $R_1 = H$) 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, $R_1 = Me$) and (IV; R = Me, $R_1 = H$), which on ring closure furnished a mixture of the ketotetrahydronaphthalenes (V; R = H, $R_1 = Me$) and (V; R = Me, $R_1 = H$). The latter compounds on reduction gave only one product (VI).

(III.)
$$(3:4)(OMe)_2C_6H_3\cdot CO\cdot CHR\cdot CHR_1\cdot CO_2H \longrightarrow \cdot CH_2\cdot CHR\cdot CHR_1\cdot CO_2H$$
 (IV.)

$$\begin{array}{c} CH_2 \\ MeO \\ MeO \end{array} \longrightarrow \begin{array}{c} CHR \\ CHR_1 \\ CO \end{array} \longrightarrow \begin{array}{c} CH_2 \\ CH_2 \\ CH_2 \end{array} \longrightarrow \begin{array}{c} CHMe \\ CH_2 \\ CH_2 \end{array} \longrightarrow \begin{array}{c} (VII.) \end{array}$$

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 synthetical 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

picropodophyllin are represented by the modifications (XI) and (XII) of formulæ (I) and (II) respectively.

Since α - and β -apopicropodophyllin yield the same apopicropodophyllic acid (XIII), we consider that these compounds are geometrical and not structural isomerides.

EXPERIMENTAL.

 α - and β -apoPicropodophyllin.—In addition to the methods of prepn. already described, it was found that the addition of a drop of piperidine to a boiling solution of podophyllotoxin (5 g.) in MeOH (25 c.c.) and H₂O (10 c.c.) caused an almost immediate separation of picropodophyllin, m. p. 215—216°, which had m. p. 226° after purification. The reaction does not take place in the absence of H₂O.

Conc. H_2SO_4 (0·1 c.c.) was added to a warm solution of picropodophyllin (20 g.) in Ac_2O (130 c.c.), and the mixture heated on the steam-bath for 40 min. After cooling, α -apopicropodophyllin (12·5 g.) was collected, washed with Ac_2O and then with EtOH, air-dried, and repeatedly crystallised from EtOH-AcOH and once from AcOEt, forming slender needles, m. p. $236-237^\circ$, $[\alpha]_{144}^{214} - 13\cdot2^\circ$ in CHCl₃ (c, 0·418) (Found: C, 66·5; H, 5·1. Calc. for $C_{22}H_{20}O_7$: C, 66·6; H, 5·0%). In a number of similar expts. the crude products had m. p.'s between 242° and 248°, which after several recrystns. from EtOH-AcOH fell to 234—236°. Repeated recrystn. of the crude (m. p. 242°) or of recryst. material (m. p. 236—237°) from AcOEt finally gave specimens, m. p. 244—245°, $[\alpha]_{364}^{214} - 17\cdot5^\circ$ in CHCl₃ (c, 0·4852). This compound is sparingly sol. in EtOH or acetone but readily in boiling AcOH or Ac₂O. It dissolves in cold conc. H_2SO_4 , forming a brownish-red solution which on being warmed darkens and becomes dull purple.

Treatment of podophyllotoxin (5 g.) with a mixture of Ac₂O (40 c.c.) and conc. H₂SO₄ (0·2 c.c.) at 65—70° for 10 min. gave rise to acetylpodophyllotoxin, m. p. 204° after purification, $[\alpha]_{5461}^{21}$ — 174·1° in CHCl₃ (c, 0·4260) (Späth and co-workers record m. p. 204°, and Borsche and Niemann give m. p. 179—181°).

 α -apoPicropodophyllin (2 g.; crude or purified) was heated on the steam-bath with Ac₂O (15 c.c.) and dry pyridine (5 c.c.) for 2·5 hr., and the mixture poured into H₂O (200 c.c.). Next day β -apopicropodophyllin was collected and on crystn. from EtOH formed a mass of slender needles, m. p. 216°, [α]²¹₃₄₆₁ + 117·6° in CHCl₃ (c, 0·5104), identical with a specimen prepared by means of boiling Ac₂O and AcONa (Found: C, 66·5; H, 5·4%). This compound is more sol. in EtOH, AcOH, or AcOEt than the α -isomeride, from which it can readily be distinguished by its H₂SO₄ reaction; with cold conc. H₂SO₄ it forms a pale orange solution, which on warming becomes red, then purple, and finally violet. A mixture of the α - and the β -isomeride melted at 203—208°.

apo $Picropodophyllic\ Acid.$ — α -apo $Picropodophyllin\ (2.5 g.)$, m. p. 246°, was refluxed with EtOH (10 c.c.), H₂O (10 c.c.), and NaOH (2.5 g.) on the steam-bath for 20 min., diluted with H₂O (50 c.c.), cooled to 0°, and acidified (Congo-red) with cold dil. HCl. apoPicropodophyllic acid thus pptd. was isolated with CHCl₃ and on crystn. from C₆H₆ formed colourless slender needles, m. p. 174° (decomp.), $\lceil \alpha \rceil_{5461}^{212} - 279.4°$ in CHCl₃ (c, 0.9624) (Found: C, 63.7; H, 5.6. Calc. for C₂₂H₂₂O₈: C, 63.7; H, 5.4%). This compound was identical with the acid (m. p. 174°) derived from β -apopicropodophyllin, for which Borsche and Niemann record m. p. 160—165° but do not state the rotation. The H₂SO₄ reaction was identical with that of α -apopicropodophyllin.

apoPicropodophyllic acid (2 g.) was converted into the α -lactone by being heated at 185° for $\frac{1}{2}$ hr. Cryst. from AcOH, the product had m. p. 237°, alone or mixed with an authentic specimen, m. p. 236—237°. Repeated crystn. from AcOEt finally gave α -apopicropodophyllin, m. p. and mixed m. p. 244°.

Podophyllomeronic Acid.—HI (20 c.c.; d 1.7) was added to a suspension of α - or β -apopicropodophyllin (5 g.) in warm AcOH, and the mixture gently refluxed for 20 min. H₂O (5 c.c.) was then added and next day the cryst. acid (0.8 g.) was collected, washed with aq. NaHSO₃, 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.), PhNO₂ (80 c.c.), veratrole (20 g.), and anhydrous AlCl₃ (38 g.) was kept at room temp. for 50 hr. After the addition of ice (100 g.) and dil. HCl (50 c.c.) the PhNO₂ was removed in steam and the residual oil isolated with Et₂O and dissolved in aq. NaHCO₃. The filtered solution (charcoal) was acidified with conc. HCl, and the product obtained as a viscous syrup which did not solidify.

The mixed β-veratroylpropionic 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 Et₂O and purified by means of aq. NaHCO₃.

A mixture of the latter acids and 90% $\rm H_2SO_4$ (80 c.c.) was heated on the water-bath for 20 min., cooled, and poured on ice (300 g.). A CHCl₃ solution of the semi-solid product was washed with aq. Na₁CO₃, then with dil. aq. Na₂CO₃, and finally with H₂O, dried, and evaporated. Distillation of the dark residue in a high vac. gave the mixed ketones as a colourless solid; a specimen cryst. from Et₂O-light petroleum had m. p. 102—110° (Found: C, 70·6; H, 7·2. $\rm C_{13}H_{16}O_3$ 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 FeCl₃ 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 C₁₃H₁₄O₂: C, 77·2; H, 6·9%) (Borsche and Niemann record m. p. 98—100°).
- 6:7-Dihydroxy-2-methylnaphthalene.—The dimethyl ether $(2\cdot 5 \text{ g.})$ was heated (oil-bath at 135— 140°) with HI (25 c.c.; d 1·7) and Ac₂O (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. NaHSO₃; the remainder was isolated with Et₂O. Cryst. from C_6H_6 -ligroin, it formed elongated colourless prisms, m. p. 165° (Found: C, $76\cdot 0$; H, $5\cdot 6$. Calc. for $C_{11}H_{10}O_2: C$, $75\cdot 9$; H, $5\cdot 8\%$) (Borsche and Niemann give m. p. 161— 162°). The substance gives with MeOH–FeCl₃ and with EtOH–FeCl₃ deep green and pure deep blue colorations respectively; in both cases the colour changes to blue-violet on dilution with H_2O .
- $6:7\text{-}Methylenedioxy-2\text{-}methylnaphthalene}$ (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 FeCl₃ 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 H₂O, 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 $\text{C}_{12}\text{H}_{10}\text{O}_2$: C, 77·4; H, 5·4%).

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