Summary

The addition of alcohols to Δ^{16}-20-ketosteroids in alcoholic alkali to give 16-alkoxy-20-ketosteroids has been described. The configurations of the 16-alkoxy group as α and the C-17 acetyl group as β have been assigned. The reaction has been shown to be reversible, and the same equilibrium mixture has been obtained from both directions. 16α-Methoxyandrostane-3β,17β-diol was prepared in the course of the proof of structure.

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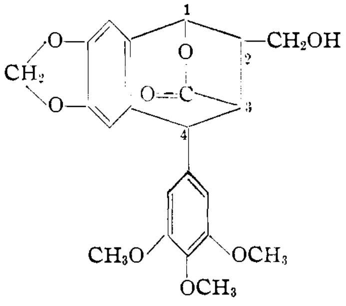
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MARYLAND]

Podophyllotoxin and Picropodophyllin.[1] I. Their Reduction by Lithium Aluminum Hydride

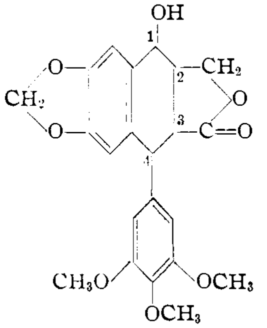
BY NATHAN L. DRAKE AND EDWARD H. PRICE[2]

Podophyllotoxin, one of the constituents of podophyllin resin, and its isomer, picropodophyllin, are of current interest because of their effect upon mitosis, an effect which is apparently shared by other constituents of the resin.[3]

The structures, I and II, for podophyllotoxin and picropodophyllin, respectively, are based upon work of Borsche and Niemann,[4] Späth[5] and Haworth.[6] Neither compound has been synthesized.



I



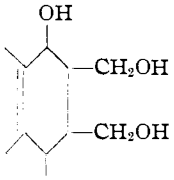
II

The carbon skeleton of the isomers is well established, but the evidence upon which the different lactone structures are based is neither sufficiently extensive nor sufficiently clear-cut to rule out other possibilities. Furthermore, no proof of the presence of a primary hydroxyl in podophyllotoxin and a secondary hydroxyl in picropodophyllin has been advanced, and the stereochemistry of the molecules has been largely neglected.

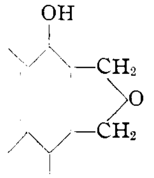
The present work was undertaken to prove whether the lactone ring of each of the isomers was essential to biological activity.

As work progressed, it became evident that podophyllotoxin and picropodophyllin must differ in configuration at carbon number 3 (see I and II). Whether there are other differences between the isomers, differences which are not expressed in the Borsche–Späth formulas, is not proven by our work, but our evidence can be satisfactorily interpreted in terms of the Borsche–Späth formulas.[7]

When podophyllotoxin is reduced by lithium aluminum hydride, a trihydroxy compound, IIIa is produced. This substance is very sensitive to acidic reagents and cannot be recrystallized due to the ease with which it undergoes loss of water. The dehydration product is formulated as IVa, although it is quite possible that the tetrahydrofuran ring might be formed through the hydroxyl group on carbon-1. IIIa is characterized by the formation of a tri-p-nitrobenzoate formed by the treatment of the alcohol with p-nitrobenzoyl chloride in pyridine. The presence of only one hydroxyl group in IVa follows from its conversion into (1) a methyl ether containing four methoxyl groups, (2) a monobenzoate and (3) a mono-p-nitrobenzoate. The molecular weight of IVa was also in accord with that calculated assuming internal loss of water from the molecule.



IIIa,b



IVa,b

We have been unable to obtain any direct chemical proof of the presence of a *secondary*

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(1) From a thesis submitted to the Graduate School of the University of Maryland by Edward H. Price in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1949.

(2) American Chemical Society predoctoral fellow 1946–1949. The junior author wishes to express his thanks to the American Chemical Society for the fellowship which made this work possible. The podophyllotoxin used was made available through a grant-in-aid (C 430 C) from the National Institutes of Health.

(3) Hartwell and Detty, THIS JOURNAL, **72**, 246 (1950).

(4) Borsche and Niemann, *Ann.*, **494**, 126 (1932); *Ber.*, **65**, 1633 (1932).

(5) Späth, *et al.*, *Ber.*, **65**, 1536, 1773 (1932); **66**, 125 (1933).

(6) Haworth and Richardson, *J. Chem. Soc.*, **149**, 348 (1935).

(7) In a paper presented before the Medicinal Chemistry Division at Philadelphia, April 10, 1950, Hartwell and Shrecker presented evidence to show that the sole difference between podophyllotoxin and picropodophyllin is the configuration around carbon-3.

hydroxyl group in IVa. The substance does not undergo hydrogenolysis when treated with palladium and hydrogen at room temperature or Raney nickel and hydrogen at 80°. Such resistance to hydrogenolysis, unusual for a substituted benzyl alcohol, is not unexpected in view of the extreme resistance of piperonyl alcohol to hydrogenolysis.[8]

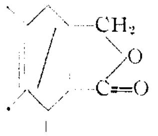
Many attempts to oxidize IVa to the corresponding ketone by the calculated amount of chromic anhydride or potassium permanganate resulted in extensive degradation and recovery of starting material. Other attempts to produce the ketone derived from IVa included Oppenauer oxidation using cyclohexanone as the hydrogen acceptor and treatment with benzoquinone in sunlight.[9]

The failure of all of our numerous experiments to convert IVa into the related ketone together with failure to dehydrogenate IVa to a naphthalene and the stability of the benzoate of IVa to pyrolysis are difficult to explain in terms of the formula written.

Picropodophyllin is very sparingly soluble in ether, and cannot be reduced readily by lithium aluminum hydride in this solvent alone as was podophyllotoxin. However, by appropriate modification of the method used for reducing podophyllotoxin, it proved possible to reduce picropodophyllin to IIIb, a diastereoisomer of IIIa. This fact is clear evidence of the existence of a difference in configuration between podophyllotoxin and picropodophyllin. This difference is interpreted as a difference in configuration around carbon-3. Inasmuch as podophyllotoxin readily isomerizes to picropodophyllin, it is tempting to conclude from this evidence that the carboxyl group is *trans* to the hydroxymethyl in podophyllotoxin and *cis* to it in picropodophyllin. However, examination of models indicates that a lactone ring should form equally readily whether the hydroxymethyl group and the carboxyl group bear a *cis* or *trans* relationship to each other. The only conclusion which seems at present to be justified by the evidence is that carbon-3 undergoes inversion in configuration when podophyllotoxin is converted into picropodophyllin.

Like its isomer, IIIa, the trihydroxy compound from picropodophyllin undergoes ready loss of water in the presence of acidic reagents to yield IVb. Nitrobenzoates from IIIb and IVb confirm the presence of the required number of hydroxyl groups in each compound. Compound IVb is monomolecular according to its molecular weight by the Rast method.

β-Apopicropodophyllin (V), has been prepared by several investigators by the dehydration of picropodophyllin.[4,5,10] We have found that pyrolysis of the benzoate of picropodophyllin produces only the β-isomer and that the yields obtainable by this method are far better than those obtainable by methods previously described.

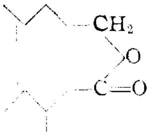


V α, β (the diagonal indicates the presence of an unlocated double bond)

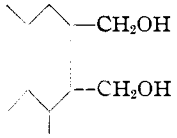
The clean conversion of the benzoate of picropodophyllin to β-apopicropodophyllin leads us to assign a *cis*-arrangement of the benzoxy group and the adjacent hydrogen.[11] Barton has reviewed the yields obtainable in reactions involving elimination of acid by pyrolysis from a number of acyloxy derivatives of compounds of the terpene series. His evidence indicates that a *cis*-configuration decidedly favors the elimination in contradistinction to the situation which obtains during the acid-catalyzed elimination of water, when the process is a *trans*-elimination.

β-Apopicropodophyllin, which proved to be surprisingly resistant to hydrogenation, was finally converted to a tetrahydronaphthalene by treatment at 60° with hydrogen and Raney nickel; the hydrogenation was very slow. Borsche and Niemann[4] have reported the preparation of desoxypicropodophyllin whose melting point was found by them to be 169–170°; our product melted at 199.8–201°.

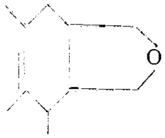
Desoxypicropodophyllin (VI) was converted by reduction by lithium aluminum hydride to VII. This substance was characterized by a di-*p*-nitrobenzoate. Compound VII underwent the same easy loss of water which characterized the behavior of IIIa and IIIb, and yielded a crystalline tetrahydrofuran derivative, VIII. Attempts to dehydrogenate IX to the corresponding naphthalene in the presence of palladium-charcoal catalyst were unsuccessful.



VI



VII



VIII

The ultraviolet absorption spectra of the various compounds prepared were studied. The extinction coefficients of the prominent maxima and minima and the wave lengths corresponding to these maxima and minima are given in Table I.

The only spectrum which is markedly different from the others is that of α-apopicropodophyllin. The very considerable shift of the maximum toward longer wave lengths in passing from podophyllotoxin to α-apopicropodophyllin is not shown by any of the other substances examined. The curves for the other spectra could be very nearly superimposed on that of podophyllotoxin.

The difference of the reduction products of podophyllotoxin and picropodophyllin can mean only that there is a stereochemical difference about one or more of the carbon atoms in the tetralin

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[8] Reeve and Sterling, THIS JOURNAL, **71**, 3657 (1949).

[9] Ciamician and Silber, *Ber.*, **34**, 1530 (1901).

[10] Robertson and Waters, *J. Chem. Soc.*, 83 (1933).

[11] Barton, *ibid.*, 2174 (1949).

TABLE I

Compound	ϵ_{max}	ϵ_{min}	Wave length, mμ	
			Max.	Min
I	4570	1260	292	259.5
II	4520	1130	289	260
IIIa	4390	1100	292.5	259
IIIb	4230	1030	292	259
IVa	5010	1460	293	263
IVb	5170	1720	293	262
V (α)	7670	3490	311	265
V (β)	4660	2830	291	268
VI	5200	1120	291	260
VII	4890	880	294	258
VIII	4810	870	293	258

nucleus, and the change or changes in configuration that occur when podophyllotoxin is converted to picropodophyllin must be confined to carbons 1 and/or 3. If a change in configuration occurred at carbon-1 due to cleavage between oxygen and carbon, racemization with respect to carbon-1 should follow. Inasmuch as the conversion of podophyllotoxin to picropodophyllin is essentially quantitative, the possibility of racemization with respect to carbon-1 appears to be excluded. It seems reasonable to conclude, therefore, that the conversion of podophyllotoxin to picropodophyllin involves at least an inversion of configuration at carbon-3. Such behavior has been reported for other compounds in the lignane series, *viz.*. matairesinol[12] and conidendrin.[13]

Structure IVb is the only one sterically possible, if we assume that the hydroxyl group at carbon-1 and the hydroxymethyl attached to carbon-3 are *trans* in the trihydroxy compound IIIb. Present evidence is not sufficient to exclude the possibility of ether formation through the hydroxyl at carbon-1 instead of through the hydroxymethyl group in IVa.

There are many conflicting reports in the literature[4,5,10] regarding melting points and rotations of several of the compounds discussed above. Certain of these have been reinvestigated; the values obtained in our laboratories are given in the experimental part.

Experimental

Podophyllotoxin (I).—The crude podophyllotoxin[14] could not be purified sufficiently for analysis by the recrystallization procedures described in the literature.[3,4] Purification was accomplished by sublimation under a pressure of 0.1 to 0.2 micron (McLeod) at a pot temperature of 105–110°. The sublimate melted at 116.8–119.5° with sintering at 114.5°. *Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.55, 63.55; H, 5.44, 5.44; $[\alpha]^{25}D$ −126°; *c*, 0.397 g./100 ml., chloroform.

Picropodophyllin (II).—Treatment of podophyllotoxin with sodium acetate in aqueous alcohol[3] gave 80% yields of picropodophyllin. After several recrystallizations of the crude substance from alcohol, fine white needles which melted at 223.5–224.5° were obtained. *Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.39, 63.46; H, 5.37, 4.99. $[\alpha]^{25}D$ 0° at *c* 0.449 g./100 ml., chloroform; $[\alpha]^{25}D$ 0° at *c*, 0.694 g./100 ml., chloroform.

α-Apopicropodophyllin (V).—A portion of the crude acetate of picropodophyllin, obtained by the action of sodium acetate and acetic anhydride on picropodophyllin, was recrystallized from acetic anhydride after evaporation nearly to dryness.[5] The product melted at 233.8–235.2° with sintering at 233°. Two further recrystallizations from an alcohol-acetic acid mixture raised the melting point to 235.4–236.3°. *Anal.* Calcd. for $C_{22}H_{20}O_7$: C, 66.65; H, 5.08. Found: C, 66.39, 66.61; H, 5.14, 5.09. On an identical sample $[\alpha]^{25}D$ −18°; *c*, 0.581 g./100 ml. chloroform. Robertson[10] has reported that cold concentrated sulfuric acid gives different characteristic color changes with alpha and beta-apopicropodophyllin. This test has been confirmed on both substances.

Trihydroxy Compound, IIIa.—Dry podophyllotoxin (2.0 g., 0.0048 mole) was placed in a Soxhlet extractor and extracted into a solution of 0.22 g. (0.0058 mole) of lithium aluminum hydride in 500 ml. of ether in a one-liter flask. The reaction mixture was stirred and protected from the atmosphere by drying tubes. At the end of 7 hours all the podophyllotoxin had been extracted into the reaction; stirring and refluxing were continued for one hour longer. At the end of that time the excess hydride was decomposed by the cautious addition of water and, when hydrogen was no longer evolved, 100 ml. of 10% sodium hydroxide was added, and the mixture stirred until the ether layer was clear. The layers were separated, and the aqueous layer was placed in a liquid-liquid extractor and the ether in its boiler. Extraction of the aqueous phase was continued for 40 hours during which time two substances separated out of the ether solution. One substance, consisting of very fine feathery needles, was later recognized as picropodophyllin resulting from unreduced podophyllotoxin. The second substance separated as hard rectangular plates and was the trihydroxy compound. The ether solution was decanted from both these substances and concentrated to 100 ml.; 0.5 g. (25%) of hard rectangular plates of the trihydroxy compound separated from the cooled solution. The compound melted on a hot-stage under the microscope at 198.5–200.5° and remelted at 198–200°. *Anal.* Calcd. for $C_{22}H_{26}O_8$: C, 63.12; H, 6.25. Found: C, 62.89, 63.20; H, 6.28, 6.28. $[\alpha]^{25}D$ 0°; *c*, 0.312 g./100 ml., chloroform.

Subsequent preparations were modified to give much improved yields. Ten grams (0.024 mole) of podophyllotoxin was placed in a Soxhlet extractor on a glass-wool plug instead of a conventional thimble. Extraction into a solution of 3.0 g. (0.078 mole) of lithium aluminum hydride in 3 liters of ether required approximately 5 hours. Heating and stirring were continued for 4 hours additional. It is very important in this reaction that stirring be very vigorous. At the end of the reaction time, excess hydride was decomposed cautiously by the dropwise addition of water, whereupon 400 ml. of 10% sodium hydroxide was added. As soon as the ether layer cleared it was decanted carefully and the alkaline layer washed by swirling and decantation with three or four successive 400-ml. portions of ether. Rapidity in separating and washing the alkaline layer is important since the trihydroxy compound often crystallizes very quickly. The combined ether solutions, after being concentrated to 700–800 ml. and cooled, yielded 7.54 g. (75%) of the trihydroxy compound, IIIa, identical with previous preparations. By washing the flasks with alcohol and combining the washings with the ether filtrates and concentrating them to 15 ml., a second crop (8%) of slightly impure material of melting point 188–194° was obtained.

Tri-*p*-nitrobenzoate of IIIa.—The tri-*p*-nitrobenzoate was prepared from IIIa by the action of *p*-nitrobenzoyl chloride in pyridine. After two recrystallizations of the product from ethyl acetate-alcohol mixture followed by recrystallization from absolute alcohol and then from an alcohol-benzene mixture, a yellow material of melting point 130.6–134.6° was obtained. Examination of the solid under the microscope failed to reveal any evidence of birefringency. *Anal.* Calcd. for $C_{43}H_{35}O_{17}N_3$: C, 59.65; H, 4.08; N, 4.85. Found: C, 59.95, 60.00; H, 4.47, 4.45; N, 5.08, 4.85. $[\alpha]^{25}D$ −31°; *c* 0.629 g./100 ml., chloroform.

Anhydro Compound, IVa.—Trihydroxy compound IIIa (2.0 g., 0.0048 mole) was digested in alcohol for 1.5 hours with 0.05 g. of *p*-toluenesulfonic acid, whereupon the solution was concentrated to 50 ml. and cooled. Crude anhydro compound (1.6 g.) precipitated out. A second crop which weighed 0.2 g. was collected from the concentrated mother liquors. Recrystallization of the combined first and second crops of crude material from alcohol gave 1.65 g. (83%) of the anhydro compound, IVa, which melted at 256.3–257.3° with sintering at 253°. *Anal.* Calcd. for $C_{22}H_{24}O_7$: C, 65.98; H, 6.04; mol. wt., 400.4. Found: C,

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[12] Haworth and Atkinson, *J. Chem. Soc.*, 797 (1938).

[13] Holmberg and Sjoberg, *Ber.*, **54**, 2406 (1921).

[14] All podophyllotoxin used in this work was purchased from S. B. Penick and Co.

65.78, 65.91; H, 6.25, 6.19; mol. wt. (Rast), 385, 409. $[\alpha]^{25}\mathrm{D}$ +13°; *c*, 0.489 g./100 ml., chloroform.

Mono-*p*-nitrobenzoate of IVa.—The mono-*p*-nitrobenzoate of IVa, prepared in pyridine, was obtained as a yellow crystalline solid which after four recrystallizations from alcohol melted at 194.7-196.1° dec. with sintering at 193°. *Anal.* Calcd. for $C_{29}H_{27}O_{10}N$: C, 63.38; H, 4.95; N, 2.55. Found: C, 63.15, 63.46; H, 5.46, 5.14; N, 2.56, 2.55. $[\alpha]^{25}\mathrm{D}$ −46°; *c*, 0.474 g./100 ml., chloroform.

Methyl Ether of IVa.—The anhydro compound, IVa, (0.55 g., 0.0014 mole) was dissolved in 100 ml. of dry boiling toluene and stirred in the presence of a small piece of sodium (approximately 0.3 g., 0.013 mole) for 24 hours. The solution was then cooled to approximately 80°, and 8 ml. (18.4 g., 0.13 mole) of methyl iodide was added slowly in 20 ml. of dry benzene as a diluent. The mixture was then heated under reflux and stirred for 3 hours, cooled to 60°, and filtered from the precipitated sodium iodide. After removal of the toluene and benzene by steam distillation, a yellow solid remained which melted at 155-165° and was very soluble in most solvents. Recrystallization from a large volume of petroleum ether (90-100°) yielded 0.4 g. (72%) of soft yellow crystals of the methyl ether, which melted at 155-169.5°. Further recrystallization did not improve the melting point. For analysis, a sample was sublimed under a pressure of 0.1-0.2 micron (McLeod) at a pot temperature of 150-155°. The sublimate retained the light yellow color, and the melting point was raised to 167.1-173.6° with sintering at 163°. *Anal.* Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.31. Found: C, 66.90, 66.89; H, 6.38, 6.45. $[\alpha]^{25}\mathrm{D}$ +3°; *c*, 0.450 g./100 ml., chloroform.

Benzoate of IV.—The benzoate of the anhydro compound, IVa, was prepared by treatment with benzoyl chloride in pyridine; after recrystallization of the crude substance from alcohol, a product was obtained (85%) which melted at 169.6-171.6°. *Anal.* Calcd. for $C_{29}H_{28}O_8$: C, 69.04; H, 5.60. Found: C, 69.05, 68.85; H, 5.91, 5.87. $[\alpha]^{25}\mathrm{D}$ −27°; *c*, 0.498 g./100 ml., chloroform.

Hydrolysis of the Benzoate of IVa to IVa.—The benzoate described directly above (0.21 g., 0.00042 mole) was dissolved in approximately 6 ml. of warm ethyl alcohol, and 0.5 ml. of 5% sodium hydroxide (0.00065 mole) was added. The solution was heated under reflux for 2 hours, then added to 10 ml. of water and cooled. The precipitated product was filtered off; there was obtained 0.17 g. (theoretical yield) of compound IVa. The melting point of the product was 256.8-258.8°, and a mixed melting point determination with IVa showed no depression.

Pyrolysis of the Benzoate of IVa.—The benzoate was completely stable to sublimation in high vacuum; therefore, 0.15 g. of the benzoate was heated under nitrogen to 250-260° for 1 hour. The cooled melt was a yellow glass, but it readily crystallized when alcohol was added. After one recrystallization there was obtained 0.11 g. (73%) of starting material of melting point 172.1-173.1°. There was no indication of extensive dehydration. Under similar conditions, the benzoate of picropodophyllin is converted smoothly to β-apopicropodophyllin.

Trihydroxy Compound, IIIb.—Picropodophyllin (10.8 g., 0.026 mole) was dissolved in 500 ml. of dry purified dioxane and added slowly over the course of 45 minutes to a vigorously stirred solution of 3.0 g. (0.078 mole) of lithium aluminum hydride in 3.5 l. of dry, alcohol-free ether boiling under reflux. Refluxing and stirring were continued for 7 hours. Excess hydride was decomposed as before with 400 ml. of 10% sodium hydroxide, and the ether layer decanted immediately. The aqueous layer was washed with several portions of ether by swirling and decantation, and the washings combined with the original ether solution. Two-thirds of this solution (equivalent to 7.2 g. of picropodophyllin) was concentrated to a volume of 350 ml., cooled and seeded with trihydroxy compound previously obtained. When crystallization did not take place then or on further concentration, the solvent was removed under diminished pressure at a temperature of 50°. The residue was taken up in ether, and the resulting solution was concentrated to 250 ml. and seeded with the trihydroxy compound IIIb. Crystallization took place immediately, and after filtration there was obtained 3.8 g. (53%) of white crystals which melted at 160.2-162.2°. *Anal.* Calcd. for $C_{22}H_{26}O_8$: C, 63.12; H, 6.25. Found: C, 63.01, 62.84; H, 6.51, 6.44. $[\alpha]^{25}\mathrm{D}$ −67°; *c*, 0.347 g./100 ml., chloroform.

The crystals of IIIb necessary for seeding purposes were obtained by allowing a portion of the crude reduction product of picropodophyllin prepared in a manner similar to the above to stand for about 7 weeks. Crystals of the trihydroxy compound IIIb finally formed and were triturated with ether and removed by filtration.

Tri-*p*-nitrobenzoate of IIIb.—The tri-*p*-nitrobenzoate of IIIb was prepared from a crude, non-crystalline reduction product of picropodophyllin obtained in a manner similar to that described directly above, but with the use of tetrahydrofuran as solvent. The preparation of the benzoate was carried out in pyridine, and the resultant yellow solid was recrystallized by dissolving it in benzene and pouring the benzene solution into a large volume of boiling alcohol. Concentration of the benzene-alcohol solution removed most of the benzene, and when cloudiness appeared, the solution was chilled rapidly whereupon a soft yellow precipitate of the triester separated. After several recrystallizations, the melting range was constant at 118.5-125.5°, within which temperature range a thick glassy melt was obtained. Examination of the triester under the microscope gave only slight indications of birefringency. *Anal.* Calcd. for $C_{43}H_{35}O_{17}N_3$: C, 59.65; H, 4.08; N, 4.85. Found: C, 59.77, 59.60; H, 4.59, 4.30; N, 5.08, 4.83. $[\alpha]^{25}\mathrm{D}$ −29°; *c*, 0.942 g./100 ml., chloroform; $[\alpha]^{25}\mathrm{D}$ −27°; *c*, 0.377 g./100 ml., chloroform.

Anhydro Compound IVb.—Six hundred ml. (equivalent to 1.0 g. of picropodophyllin) of an ether-dioxane solution, from a lithium aluminum hydride reduction of picropodophyllin was concentrated to 40 ml., whereupon 150 ml. of benzene was added. Solvent was then removed by fractional distillation up to the boiling point of benzene. *p*-Toluenesulfonic acid (0.05 g.) was then added and the resultant solution was heated under reflux for 28 hours with the returning solvent passing through a tube of Drierite. After evaporation of the solvent, there remained 0.65 g. (65%) of a slightly colored glass, which could not be induced to crystallize. *Anal.* Calcd. for $C_{22}H_{24}O_7$: C, 65.98; H, 6.04. Found: C, 65.89, 66.17; H, 6.43, 6.25. $[\alpha]^{25}\mathrm{D}$ +73°; *c*, 0.463 g./100 ml., chloroform. Molecular weight determinations by the Rast method gave values of 454 and 459, which though not as close as desirable to the theoretical value of 400.4, clearly demonstrate that the compound is not dimeric. Examination of the anhydro compound, IVb, under the microscope failed to give any indication of birefringency.

Mono-*p*-nitrobenzoate of IVb.—The mono-*p*-nitrobenzoate was prepared in pyridine, and after several recrystallizations from a large volume of alcohol was obtained as a light yellow solid which melted to a clear thick oil at 90-94.9°. Examination of the solid under the microscope failed to reveal any indication of a crystalline nature. *Anal.* Calcd. for $C_{29}H_{27}O_{10}N$: C, 63.38; H, 4.95; N, 2.55. Found: C, 63.60, 63.39; H, 5.35, 5.15; N, 2.81, 2.80. $[\alpha]^{25}\mathrm{D}$ +66°; *c*, 0.197 g./100 ml., chloroform.

Benzoate of Picropodophyllin.—Picropodophyllin (5.3 g., 0.0012 mole) was dissolved in 70 ml. of dry pyridine and 4 ml. of benzoyl chloride (0.034 mole) was added. The solution was heated on a steam-bath for one hour and then concentrated to about 35 ml. This solution was added to 200 ml. of water, cooled, and filtered. The crude benzoate after drying weighed 6.5 g. (98%) and melted at 185-192°. Two recrystallizations from ethyl acetate raised the melting point to a constant value of 200.1-201.6°. *Anal.* Calcd. for $C_{29}H_{26}O_9$: C, 67.17; H, 5.05. Found: C, 66.99, 66.98; H, 5.32, 5.31. $[\alpha]^{25}\mathrm{D}$ +13°; *c*, 0.569 g./100 ml., chloroform.

Benzoate of Podophyllotoxin.—Podophyllotoxin (2.0 g., 0.0048 mole) was dissolved in 20 ml. of pyridine and 1.6 ml. of benzoyl chloride (0.014 mole) was added. The solution was allowed to stand for 15 minutes at room temperature and then poured into 80 ml. of water and cooled; the oil which formed was separated. Two recrystallizations from alcohol, in which the ester is quite soluble, gave 1.75 g. (71%) of fine white needles which melted at 90-100°. Two further recrystallizations yielded 1.10 g. of the benzoate which sintered at 109° and melted at 112.6-116.6°. Further recrystallizations did not improve the melting point. *Anal.* Calcd. for $C_{29}H_{26}O_9$: C, 67.17; H, 5.05. Found: C, 67.26, 67.40; H, 5.11, 5.10. $[\alpha]^{25}\mathrm{D}$ −118°; *c*, 0.486 g./100 ml., chloroform.

β-Apopicropodophyllin.—This compound was prepared in three ways, (a) by the decomposition of the acetate of picropodophyllin,[3,4] (b) by the pyrolysis of the benzoate of picropodophyllin, and (c) by the pyrolysis of the benzoate of podophyllotoxin. Only method (b) which is much to be preferred will be described.

The crude benzoate of picropodophyllin (4.9 g.) was heated under nitrogen. At a pot temperature of 210° the mass melted, whereupon the temperature was raised to 240° and held at this level for 20 minutes. During this time a sublimate of benzoic acid appeared above the melt. The tube was cooled to room temperature, washed with three 5-ml. portions of 5% sodium bicarbonate, and then with copious amounts of water, and finally with one small portion of alcohol. Digestion of the product with 250 ml. of ethyl acetate until the substance had dissolved, followed by concentration of the solvent to 40 ml. and cooling, caused precipitation of 3.2 g. (85%) of pure β-apopicropodophyllin. The filtrate was concentrated to 10 ml., and 0.5 g. of additional material obtained. Recrystallization of the second crop material from ethyl acetate yielded 0.4 g. (10%) of pure compound, making the total yield 95%. A sample of β-apopicropodophyllin after sublimation for analysis melted at 214.0-215.4° with sintering at 212.8°. *Anal.* Calcd. for $C_{22}H_{20}O_7$: C, 66.65; H, 5.08. Found: C, 66.45, 66.32; H, 5.17, 5.21. Best yields were obtained by this procedure when the temperature did not exceed 250°, and the period of heating was not in excess of 20 minutes.

The yield of β-apopicropodophyllin from the pyrolysis of the benzoate of podophyllotoxin was less satisfactory (65%).

Desoxypicropodophyllin (VI).—β-Apopicropodophyllin (5.6 g.) was reduced in 500 ml. of absolute alcohol at 60° over Raney nickel for 8 hours at 1600 p.s.i. of hydrogen. The solution and washings were filtered free of catalyst and concentrated to 200 ml. and cooled, whereupon fine white needles of desoxypicropodophyllin separated. The yield was 4.3 g. (77%) of a product which melted at 200.0-201.4° and was identical with a previously prepared analytical material. The filtrate was concentrated to 30 ml. and cooled to yield a small quantity (0.15 g.) of impure desoxypicropodophyllin which melted at 182.7-191.5° with sintering at 180°.

A previous preparation had given material which melted at 199.8-201.0°. Analysis had been made on this material. *Anal.* Calcd. for $C_{22}H_{22}O_7$: C, 66.30; H, 5.57. Found: C, 66.25, 66.39; H, 5.72, 5.67. $[\alpha]^{25}_{D}$ −114°; *c*, 0.498 g./100 ml., chloroform.

Dihydroxy Compound, VII.—Desoxypicropodophyllin (1.0 g., 0.0025 mole) was placed in a Soxhlet extractor and extracted into a well-stirred solution of 0.50 g. (0.015 mole) of lithium aluminum hydride in 500 ml. of ether. After 6 hours the excess hydride was decomposed by the dropwise addition of water; 70 ml. of 15% sodium hydroxide was then added. The reaction mixture was worked up in a manner similar to that described for the other reductions. Evaporation of an ether solution of the reaction product to dryness under diminished pressure yielded a glassy residue which could not be induced to crystallize. The yield was 0.80 g. (79%). *Anal.* Calcd. for $C_{22}H_{26}O_7$: C, 66.65; H, 6.51. Found: C, 65.38, 65.39; H, 6.77, 6.79. $[\alpha]^{25}_{D}$ +120°; *c*, 0.544 g./100 ml., chloroform.

Di-*p*-nitrobenzoate of VII.—The di-*p*-nitrobenzoate was prepared as were the other nitrobenzoates above; the product after several recrystallizations from alcohol was a bright yellow solid which melted at 97-107° to form a thick clear melt. *Anal.* Calcd. for $C_{36}H_{32}O_{13}N_2$: C, 61.72; H, 4.60; N, 4.00. Found: C, 61.77, 61.97; H, 4.73, 4.69; N, 4.09, 4.10. $[\alpha]^{25}_{D}$ +59°; *c*, 0.472 g./100 ml., chloroform.

Desoxyanhydro Compound, VIII.—Compound VII (0.29 g., 0.00072 mole) was dissolved in 50 ml. of dry benzene and 0.02 g. (0.00011 mole) of *p*-toluenesulfonic acid was added. The solution was heated under reflux for 24 hours with the returning solvent passing through a tube of Drierite. After cooling, the solution was washed with three 5-ml. portions of 5% sodium hydroxide, and then with water until the washings were neutral. The benzene was dried over Drierite, filtered and evaporated to near dryness. The desoxyanhydro compound crystallized in fine white needles which melted at 162.7-164.1° with sintering at 160°. The yield was 0.21 g. (75%). The desoxyanhydro compound was very soluble in most solvents, but could be recrystallized from methyl alcohol-water mixtures of cyclohexane. Recrystallization from a methyl alcohol-water mixture gave a product which melted at 162.7-163.7° with sintering at 161.7°. *Anal.* Calcd. for $C_{22}H_{24}O_6$: C, 68.73; H, 6.30. Found: C, 69.08, 68.95; H, 6.41, 6.41. $[\alpha]^{25}_{D}$ +64°; *c*, 0.497 g./100 ml., chloroform.

Summary

1. Podophyllotoxin and picrodophyllin have been reduced by lithium aluminum hydride to diasteroisomeric trihydroxy compounds.

2. The formation of these diastereoisomers is interesting as proof that the configuration at carbon-3 of the substituted tetralin nucleus of podophyllotoxin undergoes inversion during conversion of podophyllotoxin to picropodophyllin.

3. The ultraviolet absorption spectra of a number of compounds related to podophyllotoxin and picropodophyllin have been determined.

4. An improved method of preparing β-apopicropodophyllin is described.

5. Anhydro products related to the hydroxy compounds formed by lithium aluminum hydride reduction of podophyllotoxin, picropodophyllin and desoxypicropodophyllin are described.

COLLEGE PARK, MD.

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