sable and are precipitable by alcohol. On re-chromatography, the fractions studied still possess their original chromatographic properties. Individual fractions from pneumococcal transforming DNA[11] show considerable biological activity, comparable to that present in the original preparation.

These chromatographic analyses provide further evidence for the heterogeneous nature of DNA from single sources, and the method appears applicable to the fractionation of RNA. It furnishes a technique for an experimental approach to the study of the metabolism and the character of biologically active nucleic acids.[12]

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(11) Unpublished observations, in collaboration with R. D. Hotchkiss, D. J. Hutchison and M. T. Dowling, on pneumococcal DNA possessing transforming activity.

(12) We gratefully acknowledge the valuable advice and support of Dr. G. B. Brown.

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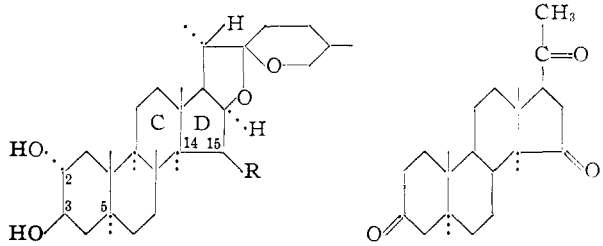
CORRELATION OF DIGITOGENIN WITH PROGESTERONE

Sir:

Although it has now been demonstrated[1,2] that digitogenin is a 2α,3β,15-trihydroxy-5α-spirostan derivative, the available evidence does not permit an unequivocal assignment to the stereochemistry of the C/D ring juncture. Thus, digitogenin (I), as the 2,3-diacetate[3] or 2,3-dicathylate[4] can be oxidized to the corresponding 15-ketone which is very readily inverted at C-14 by base. From the course of the Wolff–Kishner reduction[3] of both isomers, which proceeded in poor yield to furnish gitogenin (II), it was suggested tentatively that digitogenin has the 14β(C/D *cis*) configuration, while the opposite conclusion might be reached on the basis of the results of desulfurization studies.[4] We have now been able to arrive at a rigorous solution of this problem, which also has an important bearing on the relative stability of fused hydrindanone systems.

Δ²-5α,22a-Spirosten-15β-ol, readily obtainable[3] by sodium iodide treatment of digitogenin 2,3-dimesylate, was oxidized with perbenzoic acid to the 2α,3α-epoxide (m.p. 188–190°, $[\alpha]^{23}D$ −56° ($CHCl_3$); found: C, 75.31; H, 9.83) which was reduced with lithium aluminum hydride to 22a,5α-spirostane-3α,15β-diol (m.p. 238–240°, $[\alpha]_D$ −74° ($CHCl_3$); found: C, 75.23; H, 10.02). Side chain degradation[2] produced Δ¹⁶-allopregnene-3α,15β-diol-20-one diacetate (m.p. 142–143°, $[\alpha]_D$ −152° ($CHCl_3$), λ_{max}^{EtOH} 231 mμ, log ε 4.00; found: C, 71.74; H, 8.70) which was hydrogenated (palladium–charcoal, ethyl acetate) and saponified[5] (2% methanolic potassium hydroxide, steam bath, 2 hours) to yield allopregnane-3α,15β-diol-20-one (III), (m.p. 239–241°, $[\alpha]_D$ +59° ($CHCl_3$), +84° (pyridine), no high selective ultraviolet absorption; found: C, 75.37; H, 10.30). Mild oxidation (15 min.) with chromium trioxidel ed to allopregnane-3,15,20-trione (IV) (m.p. 222–223°, $[\alpha]_D$ +137° ($CHCl_3$); found: C, 76.72; H, 8.79), which was also obtained from 15β-hydroxyprogesterone[6] by catalytic hydrogenation (palladium–$BaSO_4$, ethyl acetate) to allopregnan-15β-ol-3,20-dione (m.p. 256–258°, $[\alpha]^{23}D$ +93° ($CHCl_3$); found: C, 76.12; H, 9.70) followed by mild chromium trioxide oxidation, or by palladium hydrogenation of 15-ketoprogesterone[6] (m.p. 155–157°, $[\alpha]^{23}D$ +200° ($CHCl_3$), found: C, 76.90; H, 8.57). Identity of the 3,15,20-trione IV, prepared by all three routes was demonstrated by infrared comparison as well as by conversion at identical rates (mutarotation: +130° → +55°), to the 14β,17α-isomer (m.p. 186–189°, $[\alpha]^{23}D$ +60° ($CHCl_3$); found: C, 76.32; H, 8.91), when allowed to stand at room temperature in 0.02 *N* methanolic potassium hydroxide for 18 hours. This latter isomerization parallels that of 15-ketoprogesterone to its 14β,17α-isomer (m.p. 213–214°, $[\alpha]^{23}D$ +113° ($CHCl_3$); found: C, 76.53; H, 8.72).[6,7]



I, R = OH
II, R = H

IV

The above correlation of digitogenin (I) with a microbiological oxidation product of progesterone establishes the 14α-configuration for digitogenin, which can now be given the rigorous name 22a,25a,-5α-spirostane-2α,3β,15β-triol.[8] It is instructive to note that while 15-keto derivatives in the choles-

(1) F. L. Warren and P. A. S. Canham, *Chem. and Ind.*, 727 (1954).

(2) C. Djerassi and T. T. Grossnickle, *ibid.*, 728 (1954).

(3) C. Djerassi, T. T. Grossnickle and L. B. High, *ibid.*, 473 (1955).

(4) D. L. Klass, M. Fieser and L. F. Fieser, THIS JOURNAL, **77** in press (1955); we are grateful to these authors for an advance copy of their paper.

(5) That no inversion occurred at C-17 had already been demonstrated with the corresponding 2α,3β,15-triacetoxy-20-ketone (ref. 2), which was regenerated after saponification and reacetylation.

(6) The microbiological oxidation of progesterone to 15β-hydroxyprogesterone has been reported by J. Fried (AAAS Gordon Research Conference on Steroids, August, 1953), *cf.* J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progress Hormone Research*, **11**, 157 (1955); J. Fried, R. W. Thoma, P. Grabowich and J. R. Gerke, *Chem. and Ind.*, in press (1955).

(7) The alkali-isomerized 3,15,20-triketones are formulated as 17α-pregnane derivatives since ring C/D *cis*-fused 20-ketosteroids possessing the 17β-configuration are epimerized at C-17 by alkali (*cf.* R. C. Elderfield, *J. Biol. Chem.*, **113**, 631 (1936); K. Meyer, *Helv. Chim. Acta*, **30**, 1976 (1947)), while those having the 17α-configuration are stable in that medium (*cf.* P. A. Plattner, H. Heusser and A. Segre, *ibid.*, **31**, 249 (1948)).

(8) The assignment of the β-configuration to the 15-hydroxyl group in III and therefore also in I is based on the following considerations: The molecular rotation values calculated for the 15α- and 15β-epimers of allopregnane-3α,15-diol-20-one from the values for 15α- and 15β-hydroxyprogesterone (*cf.* ref. 6) and the average value for the change Δ⁴-3-ketone → allo-3α-ol (−261°) (*cf.* D. H. R. Barton and W. Klyne, *Chem. and Ind.*, 755 (1948)) are +461° and +237°, respectively. The latter value (15β) is in good agreement with the found value of +197°.

tane or ergostane series are more stable in the 14α-configuration (C/D *trans*),[9] the reverse stability relationship (14$\alpha \rightarrow$ 14β) exists in 15-ketones of the spirostan series[3,4] and in 15,20-diketones of the pregnane series. It is obvious that subtle stereochemical effects can completely alter the relative stabilities of such fused hydrindane systems and that at the present time each case should be examined rigorously, rather than basing stereochemical assignments on analogy.

The smooth side chain degradation[2] of digitogenin offers a path to a variety of 15-hydroxylated steroids and experiments are now under way to prepare steroidal 15-ketones with different C-17 substituents in order to determine the effect of size and type of such substituents upon the stability of the C/D ring structure.

Two of us (C. D. and L. B. H.) are indebted to the American Cancer Society through the Committee on Growth of the National Research Council) for a research grant.

(9) C. S. Barnes, D. H. R. Barton and C. G. Laws, *Chem. and Ind.* 616 (1953).

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PODOPHYLLOTOXONE, PICROPODOPHYLLONE, AND DEHYDROPODOPHYLLOTOXIN[1]

Sir:

Assignment of a secondary alcohol grouping at the 1-position of podophyllotoxin (Ia) rests on the formation of stereoisomers in, and on the relative ease of, displacement reactions at the alcohol-bearing carbon.[2] The production of ketones (II) corresponding to podophyllotoxin and to picropodophyllin[3] now makes direct corroboration of this assignment possible.

Treatment of podophyllotoxin (Ia) with freshly prepared manganese dioxide[4] furnished (58%) the oxidation product, podophyllotoxone (IIa), m.p. 191–192° (softens 187°) and $[\alpha]^{25}D$ −125°. *Anal.* Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 64.0; H, 5.0. Ultraviolet absorption maxima were observed at 235, 277 and 316 mμ (log ϵ 4.64, 4.09 and 3.96). Peaks in the infrared at 5.62 and 5.99 μ corresponded to lactone and ketone carbonyls. No hydroxyl absorption was evident. Manganese dioxide oxidation of picropodophyllin (Ib) gave (57%) picropodophyllone (IIb), m.p. 153–154° and $[\alpha]^{25}D$ −142°. Anal. Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 64.1; H, 5.0. Maxima appeared at 240, 279 and 324 mμ (log ϵ 4.42, 3.95 and 3.91) in the ultraviolet, and at 5.66 μ and 5.98 μ—but not in the 2.5–3.0 μ region—in the infrared. The ultraviolet absorption curves of IIa and IIb, as well as of synthetic keto-ester IV[5] (λ_{max} at 236, 275 and 320 mμ, and log ϵ 4.45, 3.94 and 3.87) although differing somewhat in position and extinction of their respective maxima, are quite similar in general contour. Likewise the 5.95 μ ketone carbonyl absorption of keto-ester IV falls close to the corresponding absorption in IIa and IIb.

No rearrangement occurred in the manganese dioxide oxidations, since reduction of the two ketones, IIa and IIb, with zinc borohydride[6] regenerated the respective starting materials, podophyllotoxin (94%) and picropodophyllin (35%).

Dehydrogenation of podophyllotoxone or of picropodophyllone with selenium dioxide furnished the same product, dehydropodophyllotoxin (III) melting (in evacuated sealed capillary) at 286–288° (dec.) with preliminary softening at 275°. The melting point behavior depended on the rate of heating. *Anal.* Calcd. for $C_{22}H_{18}O_8$: C, 64.39; H, 4.42. Found: C, 64.2; H, 4.4. Absorption maxima were evident at 226, 263, 323 and 356 mμ (log ϵ 4.49, 4.62, 4.02 and 3.72), and in the infrared at 2.91 and 5.69 μ. Although this α-naphthol derivative III gave no color with ferric chloride and was not soluble in 10% sodium hydroxide solution, the presence of hydroxyl was substantiated by formation of an acetate, m.p. 259–260° (dec.) with softening at 258°. *Anal.* Calcd. for $C_{24}H_{20}O_9$: C, 63.71, H, 4.46. Found: C, 63.7, H, 4.6.

The above conversions show unequivocally that the hydroxy group in podophyllotoxin and picropodophyllin (Ia and b) cannot be tertiary, and therefore cannot be at the 2, 3 or 4 positions. Also, since manganese dioxide oxidation occurs smoothly only with allylic or benzylic alcohols,[7] it follows that the free hydroxy group must be placed on the 1 position as in I, and not on the exocyclic position as in V. The facts that the ultraviolet absorptions of compounds IIa, IIb and IV are similar, and that compounds IIa and IIb can be oxidized to naphthol III provide further support for I as opposed to V, for these facts indicate that carbonyl compounds IIa and IIb are *ketones* and not aldehydes.

Kofod and Jørgensen[8] recently isolated a compound from podophyllin, which they named "dehydropodophyllotoxin," and to which they assigned the structure of compound III. Dr. Kofod

(1) This work has been supported by grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **73**, 2909 (1951).

(3) Earlier attempts to prepare these ketones have not been rewarding; see footnote 19 of ref. 2.

(4) *E.g.*, podophyllotoxin (5.0 g.) is exposed to manganese dioxide (30 g.) in boiling chloroform (300 ml.) for one and one-quarter hours. Solvent is removed from the filtrate, and the residue is crystallized from benzene. *Cf.* D. L. Turner, THIS JOURNAL, **76**, 5173 (1954); J. Attenburrow, *et al.*, *J. Chem. Soc.*, 1094 (1952).

(5) W. J. Gensler, C. S. Samour and Shih Yi Wang, THIS JOURNAL, **76**, 315 (1954).

(6) *Cf.* E. Wiberg and W. Henle, *Z. Naturforsch.*, **7b**, 579 (1952); G. D. Barbaras, *et al.*, THIS JOURNAL, **73**, 4585 (1951); E. Wiberg, *Angew. Chem.*, **65**, 16 (1953). Ether-soluble zinc borohydride, which can be prepared from sodium borohydride and zinc chloride, may prove of special value in the reduction of alkali sensitive compounds. In our work, for example, reduction with sodium borohydride was not clean cut, and gave a product of as yet undetermined structure from both IIa and IIb.

(7) S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, **42**, 516 (1948); F. Sondheimer, C. Amendolla and G. Rosenkranz, THIS JOURNAL, **75**, 5930 (1953); M. Harfenist, A. Bavley and W. A. Lazier, *J. Org. Chem.*, **19**, 1608 (1954).

(8) H. Kofod and Chr. Jørgensen, *Acta Chem. Scand.*, **8**, 1296 (1954).