After crystallization from acetone–petroleum ether (b.p. 60–70°) the material melted at 204–205.5°; $[\alpha]_D$ +90°; λ_{max} 240 mμ (ε 16,900); (reported[6e] m.p. 201–202.5°, $[\alpha]_D$ +88°).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.16; H, 8.05.

16β-Hydroxytestosterone (IV) *from 16-ketotestosterone* (II). To a solution of 0.50 g. (1.65 mmole) of 16-ketotestosterone in 100 ml. of ethanol, cooled to 4° in an ice bath, was added a solution of 0.10 g. (2.64 mmole) of sodium borohydride in 5 ml. of water. The resulting solution was allowed to stand at 0–5° for 1 hr. Then 1.00 ml. of glacial acetic acid was added to decompose the excess sodium borohydride, and the resulting solution was evaporated to dryness. Attempts at purification of the product by direct crystallization from dilute acetone, acetone–petroleum ether (b.p. 60–70°) and dilute acetone, gave material melting at 173.5–175°. Acetylation of this material plus material recovered from the mother liquors from the crystallizations (0.382 g. total) with acetic anhydride (5 ml.) in pyridine (5 ml.) yielded, after crystallization from dilute acetone, then acetone–petroleum ether (b.p. 60–70°), 204 mg. of 16β-hydroxytestosterone diacetate (V), m.p. 198–203°. A final crystallization from acetone–petroleum ether (b.p. 60–70°) gave material, m.p. 203–204.5°, $[\alpha]_D$ + 89°, that was identical in all respects (m.m.p. and infrared spectra) with that prepared above.

Hydrolysis of 153 mg. of 16β-hydroxytestosterone diacetate (V) with 0.20 g. of sodium hydroxide in 1 ml. of water and 7 ml. of methyl alcohol under nitrogen (room temperature, 18 hr.) gave, after crystallization from dilute methanol, 96.5 mg. (81%) of 16β-hydroxytestosterone, m.p. 180–182.5°. After crystallization from dilute acetone, this material, m.p. 183–184.5°, proved to be identical in all respects (m.m.p. and infrared spectra) with that obtained directly from the fermentation.

16β-Hydroxytestosterone acetonide was prepared by treating a solution of 16β-hydroxytestosterone (46 mg.) in 5 ml. of acetone with *p*-toluenesulfonic acid (0.10 g.) for 18 hr. The acid was neutralized with solid potassium carbonate. The product was precipitated with water and crystallized from dilute acetone, m.p. 183.5–187° (cloudy melt); $\lambda_{max}^{CHCl_3}$ 5.98, 6.18, 7.22, 7.26, 9.42, and 11.53 μ.[11]

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.98; H, 9.25.

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(11) S. A. Barker, E. J. Bourne, R. M. Pinkard, and D. H. Whiffen, *J. Chem. Soc.*, 807 (1959). H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, 1958 (1957).

Communications to the Editor

High Pressure Gas Chromatography above Critical Temperatures[1]

Sir:

In studies on the separation of porphyrin mixtures by conventional gas chromatography, sufficiently high vapor pressures were reached only at temperatures where decomposition occurred. Higher vapor concentrations at lower temperatures are necessary for separations. For thermodynamic reasons liquids show higher vapor tension under pressure from insoluble gases.[2-4] This increase is relatively small. Inorganic and organic solids,[3-9] including a derivative of chlorophyll,[5] are soluble in carbon dioxide, ammonia, sulfur dioxide, ethanol, and ether far in excess of the thermodynamic increase in vapor tension. Solubility increases with pressure and van der Waals' interaction in the gas. Later the dissolution of polymers was found to be common.[10]

In our apparatus (see Fig. 1) the gas is contained in a pressure vessel and passed under 800 to 2300 p.s.i. pressure through a spiral of copper tubing for temperature equilibration, then through the chromatographic column which is made of glass, enclosed in a pressure tube and easily removable. The apparatus is mounted in an insulating box lined with heating foil. A propeller circulates the air. Thermocouples are inserted into the entrance of the column. In one modification a high pressure gauge glass was substituted for part of the pressure tube allowing visual inspection during runs. After applying the porphyrins and charging the pressure vessel the apparatus is heated above the critical temperature. Then the gas flow is started and the pressure raised to the desired level. The rate of flow is determined at ambient pressure with a pneumatic trough at the outlet.

(1) Porphyrin Studies XX. Paper XIX, E. Klesper, A. H. Corwin and P. K. Iber, *Anal. Chem.* **33**, 1091 (1961). Acknowledgment is made to the donors of the Petroleum Research Fund administered by the American Chemical Society for support of the research.

(2) W. M. Clark, *Topics in Physical Chemistry*, Williams and Wilkins, Baltimore, 1952, second ed., pp. 89–92.

(3) Walter Bueche in Houben-Weyl, *Methoden der Organischen Chemie*, Georg Thieme Verlag, Stuttgart, 1959, Vol. 1, Part 2, p. 455.

(4) F. Pollitzer and E. Strebel, *Z. physik. Chem.*, **110**, 768 (1924).

(5) J. B. Hannay and James Hogarth, *Chem. News*, **41**, 103 (1880).

(6) P. Villard, *Z. physik. Chem.*, **23**, 373 (1897).

(7) M. Centnerszwer, *Z. physik. Chem.*, **46**, 427 (1903).

(8) A. Smits, *Z. Elektrochem.*, **9**, 663 (1903).

(9) H. S. Booth and R. M. Bidwell, *Chem. Revs.*, **44**, 447 (1949).

(10) P. Ehrlich and E. B. Graham, *J. Polymer Sci.*, **45**, 246 (1960).

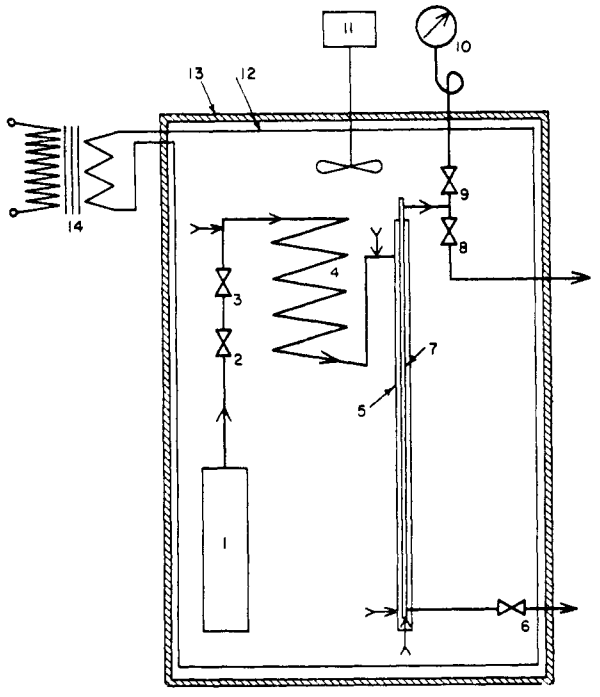

Fig. 1. High pressure gas chromatograph, schematic, 1, pressure vessel; 2, 3, 8, 9, valves; 4, spiral of copper tubing; 5, pressure tube; 6, relief valve; 7, chromatographic column; 10, gauge; 11, stirrer; 12, lining of heating foil; 13, insulating box; 14, transformer; arrows indicate location of thermocouples

To test the suitability of a gas for chromatography, a solution of 1–2 mg. of Ni etioporphyrin II or etioporphyrin II was dried on glass beads and applied to the column packed with 60–80 mesh, untreated glass beads. In several experiments each with dichlorodifluoromethane, c.t. 112°, monochlorodifluoromethane, c.t. 96°, monochlorotrifluoromethane, c.t. 28.9°, trifluoromethane, c.t. 26°, and nitrogen at a maximum pressure of 2000 p.s.i. and 150–170°, only the first two gases removed the porphyrins from the column. Even the first gas showed no effect from 1–600 p.s.i. Above 1000 p.s.i. with the first and 1400 p.s.i. with the second, vaporization increased with increasing gas pressure. The porphyrins could be recovered at the outlet valve.

The column, 30 inches long, was packed with a stationary phase of 33% polyethyleneglycol (Carbowax 20 M, Union Carbide) on Chromosorb W, 60–80 mesh, as inert support. (An experiment with Woelm alumina gave no movement, showing the need for the usual partition effect of gas chromatography.) A mixture of 1 mg. Ni etioporphyrin II and 1 mg. Ni mesoporphyrin IX dimethylester dissolved in 0.2 cc. *o*-dichlorobenzene was applied to a plug of glass wool in front of the packing but not in contact with it. One typical experiment with dichlorodifluoromethane had these characteristics: 1830 p.s.i. at start, 1660 p.s.i. at time when valve to pressure vessel was closed, 50–150 cc./min. flow rate, 60 min. duration, release of pressure from 1660 p.s.i. at approximately 300 cc./min., separated band of Ni etioporphyrin II was located 8 inches from the beginning of packing as measured from the center of band, width of band 3 inches, band of Ni mesoporphyrin IX dimethylester was located 3 inches from beginning, width $1^7/_8$ inches. X-ray powder patterns showed the components unchanged; recovery was nearly quantitative. The speed of development increased with pressure and flow rate. Monochlorodifluoromethane could be used in place of dichlorofluoromethane but was weaker in solvent action at the same pressure. A polysebacate (Harflex 370, Harchem Division) could be substituted for polyethylene glycol.

The increase in mobility with increasing pressure suggests still lower operating temperatures. Other compounds, including some of higher molecular weight, will be investigated.

Acknowledgment. We are very grateful to Mr. Joseph Walter for building the apparatus and for frequent help.

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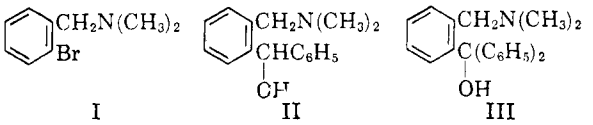
2-, 3-, and 4-Lithiobenzyldimethylamines. Grignard Reagent of 2-Bromobenzyldimethylamine[1]

Sir:

Butyllithium in ether has often been used for lithium-halogen interchanges[2] and for metalations.[3] We have observed that this reagent converts both 2-bromobenzyldimethylamine (I) and benzyldimethylamine (10 hrs., room temperature) to a lithio derivative, which reacts with benzaldehyde and benzophenone to give carbinols II and III, respectively. The yields were good to excellent.



That I and benzyldimethylamine afforded the same carbinols was established by comparisons of the infrared spectra of each carbinol obtained by the two processes and by the mixed melting

(1) Supported by the U. S. Army Research Office (Durham).

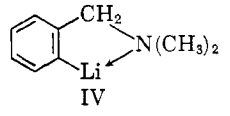
(2) R. G. Jones and H. Gilman, *Org. Reactions*, VI, 339 (1951).

(3) H. Gilman and J W. Morton, *Org. Reactions*, VIII, 258 (1954).

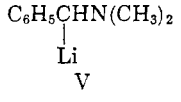
point method. Carbinol II: m.p. 71.5–72°; *Anal.* Calcd. for $C_{16}H_{19}NO$: C, 79.65; H, 7.94; N, 5.81. Found: C, 79.52; H, 8.14, N, 5.81. Carbinol III: m.p. 153.5–154°; *Anal.* Calcd. for $C_{22}H_{23}NO$: C, 83.24; H, 7.30; N, 4.41. Found: C, 83.30; H, 7.47; N, 4.36.

The structures of II and III were supported by infrared spectra. Moreover, the melting point of II agreed with that reported for this compound prepared from 2-dimethylaminomethylbenzaldehyde and phenylmagnesium bromide.[4]

The intermediate lithio derivative from I was presumably IV, and that from benzyldimethylamine was IV or possibly V. If the latter derivative were formed, its reactions with benzaldehyde and with benzophenone were accompanied by rearrangement.

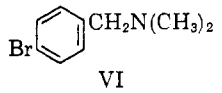


IV

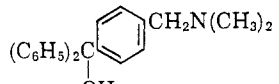


V

Similar to I, 4-bromobenzyldimethylamine (VI) was converted by butyllithium to its lithio derivative, which reacted with benzophenone to form carbinol VII, isolated as its methiodide (86%), m.p. 229–230°. *Anal.* Calcd. for $C_{23}H_{26}INO$: C, 60.12; H, 5.70; N, 3.05. Found: C, 60.14; H, 5.77; N, 2.95.



VI



VII

However, 3-bromobenzyldimethylamine (VIII) gave a mixture of products on treatment with butyllithium followed by benzophenone. The formation of the lithio intermediate may have been accompanied by the benzyne type of reaction.

These interesting types of reactions are being further investigated. The process illustrated by metalation of benzyldimethylamine should be useful in synthesis where the 2-bromo compound is not readily available.

Earlier we had observed that I affords a Grignard reagent, which reacted with benzaldehyde to give II (80%) and with several substituted benzaldehydes to form carbinols that were employed in proof of structures of products obtained from certain ortho-substitution rearrangements.[5] Attempts to prepare Grignard reagents from VI and VIII were unsuccessful.

Recently, Mehta and Zupicich[6] have similarly reported that certain 2-bromobenzyldialkylamines form Grignard reagents, whereas 3- and 4-bromobenzyldialkylamines do not.

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(4) H.-W. Bersch and R. Meyer, *Arch. Pharm.*, **287**, 613 (1954).

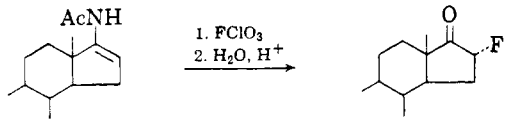
(5) F. N. Jones, Ph.D. thesis, Duke University.

(6) N. B. Mehta and J. Zupicich, Abstracts of the Division of Organic Chemistry of the American Chemical Society, Chicago, Ill., September 1961, pp. 34Q–35Q.

16α-Fluorinated Steroids from the Reaction of Perchloryl Fluoride with an Enamide

Sir:

The reaction of perchloryl fluoride with enamine,[1,2] enol ether,[3] and enol ester[4] derivatives of saturated and 4,5-unsaturated 3-ketosteroids provides a convenient method for the introduction of a fluorine substituent adjacent or vinylogous to the carbonyl group. Application of these reactions to 17-ketosteroids has been limited by the failure of these ketones to form enol ethers or enamines[5] and by the fact that in our experience enol acetate derivatives of saturated ketones are rather unreactive toward perchloryl fluoride. We now have found that an acetylated 17-amino-Δ^{16}-steroid (enamide), which is conveniently prepared[6] by the Beckmann rearrangement of the oxime of a 20-keto-Δ^{16}-steroid, reacts smoothly and stereospecifically with perchloryl fluoride to furnish the corresponding 16α-fluoro-17-ketosteroid in high yield.



(1) R. B. Gabbard and E. V. Jensen, *J. Org. Chem.*, **23**, 1406 (1958); S. Nakanishi, R. L. Morgan, and E. V. Jensen, *Chem. & Ind.*, 1136 (1960).

(2) R. Joly and J. Warnant, *Bull. soc. chim. France*, 569 (1961).

(3) S. Nakanishi, K. Morita, and E. V. Jensen, *J. Am. Chem. Soc.*, **81**, 5259 (1959).

(4) B. M. Bloom, V. V. Bogert, and R. Pinson, Jr., *Chem. & Ind.*, 1317 (1959).

(5) The only reactive 17-ketosteroid reported is 4-androstene-3,11,17-trione, which under vigorous conditions forms the 3,17-bis-*N*-pyrrolidyl enamine [(M. E. Herr and F. W. Heyl, *J. Am. Chem. Soc.*, **75**, 5927 (1953)]. We have found that this bis-enamine reacts with perchloryl fluoride to give 4,16ζ-difluoro-4-androstene-3,11,17-trione, m.p. 224–225°, α_D^{25} +174° (*c*, 1.0 in $CHCl_3$).

(6) G. Rosenkranz, O. Mancera, F. Sondheimer, and C. Djerassi, *J. Org. Chem.*, **21**, 520 (1956).

Perchloryl fluoride gas was bubbled into a solution of 2.69 g. of 3β-acetoxy-17-acetamino-5,16-androstadiene in 150 ml. of pyridine for three minutes at room temperature. After removal of excess perchloryl fluoride with a water pump, about one third of the pyridine was evaporated under reduced pressure and the residual solution poured into water, acidified to *p*H 2, and allowed to stand six hours. Extraction with ether and chromatography on Florisil gave 16α-fluoro-3β-acetoxy-5-androsten-17-one (I), 1.92 g., m.p. 198–205°, recrystallized from *n*-hexane-acetone to yield 1.76 g., 70%, m.p. 205–206°; α_D^{25} + 14° (*c*, 1.0 in $CHCl_3$); $\nu_{KBr}^{C=O}$ 1724, 1761 cm.$^{-1}$ Found: C, 72.22; H, 8.35; F, 5.43.

Acidic methanolysis of I gave 91% of 16α-fluoro-3β-hydroxy-5-androsten-17-one (II), m.p. 182–183°; α_D^{25} +18° (*c*, 0.5 in $CHCl_3$); $\gamma_{KBr}^{C=O}$ 1761 cm.$^{-1}$; Found: C, 74.23; H, 8.83; F, 6.04. Oxidation of II by the Oppenauer procedure (or by chromic acid in acetone) gave 71% of 16α-fluoro-4-androstene-3,17-dione (III), m.p. 171–172°; α_D^{25} +202° (*c*, 1.0 in $CHCl_3$); $\nu_{KBr}^{C=O}$ 1678, 1761 cm.$^{-1}$ Found: C, 74.65; H, 8.21; F, 6.10. By infrared spectrum and mixed melting point determination III was found identical with the previously reported[7] 16α-fluoro-4-androstene-3,17-dione, m.p. 171–174°, obtained with the isomeric 16β-fluoro compound from the reaction of potassium fluoride with the methanesulfonate ester of 16α-hydroxy-4-androstene-3,17-dione.

In contrast to its reaction with the enamide, the fluorination reaction of perchloryl fluoride with the enol acetate of a 17-ketosteroid is very slow and is accompanied by side reactions of chlorination. Treatment of 5,16-androstadiene-3β,17-diol diacetate in pyridine-acetone solution with perchloryl fluoride at room temperature gave no appreciable reaction in two hours, but after 20 hours it furnished 45% of crude I which could not be freed easily of chlorinated impurities. By methanolysis and Oppenauer oxidation, this crude product was converted to III, 33%, m.p. 171–172°, identical with III obtained *via* the enamide reaction.

The greatly enhanced reactivity of the enamide as compared to the enol ester is consistent with the greater ability of the nitrogen atom to donate electrons to the double bond for interaction with the electrophilic fluorine of perchloryl fluoride.

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(7) J. Fried and G. H. Thomas, U. S. Patent **2,857,403**, October 21, 1958. We are grateful to Dr. Fried for supplying us with reference samples of both isomers as well as with recent unpublished information that the configuration at position 16 has been established to be the reverse of that which had been proposed originally.

A Stereoselective Total Synthesis of *dl*-Dehydroabietic Acid

Sir:

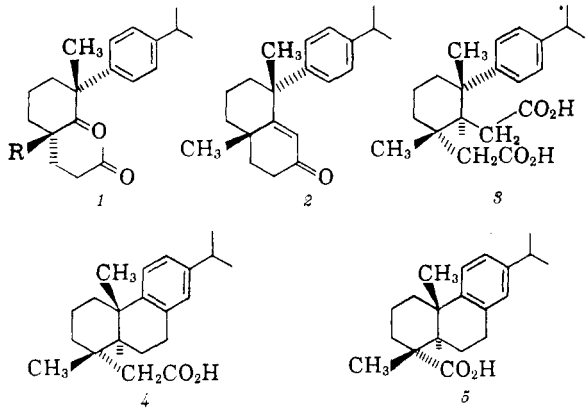
Satisfactory synthetic methods[1] have recently been developed for the elaboration of the ring C substitution pattern of the pimaric-type resin acids. An equally important phase of any program directed toward the total synthesis of these resin acids is the construction of a suitably substituted tricyclic acid to which these methods can be applied. Such an intermediate would be a derivative of dehydroabietic acid (*5*), which itself has been totally synthesized previously by Stork and Schulenberg.[2] We report here an alternative highly stereoselective total synthesis of this acid (*5*) in an over-all yield (17%) such that this synthetic scheme can be utilized for the preparation of other similar tricyclic acids of potential use in the pimaric-type resin acid synthesis.

When 6-hydroxymethylene-2-methyl-2-(*p*-isopropylphenyl)cyclohexanone [from 2-methyl-2-(*p*-isopropylphenyl)cyclohexanone[3] and ethyl formate] was treated with methyl vinyl ketone in the presence of triethylamine and then with 2% aqueous alcoholic potassium carbonate, there resulted a 67% yield of the diketone (*1*. R = H) (m.p. 51–52°; C, 79.99; H, 9.43). The less sterically hindered carbonyl on the four carbon side chain was protected as the ethylene ketal (oil; C, 76.84; H, 9.52) in 81% by treatment with ethylene glycol. When this mono ketal was methylated (potassium *t*-butoxide/methyl iodide) and the ketal cleaved (aq. hydrochloric acid/acetone), the new diketone (*2*. R = CH_3) (m.p. 54–55°; C, 80.33; H, 9.71) resulted in an 80% yield. The highly stereoselective introduction of the methyl group is in accord with the preferred axial methylation[4] of monoketal enolate; the reverse order of introduction of these substituents led equally stereoselectively to the isomeric diketone.[5]

Base-catalyzed (potassium *t*-butoxide/*t*-butyl alcohol) cyclization of the diketone (*1*. R = CH_3) afforded an 89% yield of the octalone (*2*) (m.p. 72–73°; C, 85.20; H, 9.65). The last asymmetric center of the dehydroabietic acid molecule was stereoselectively introduced in 85% yield by lithium–ammonia reduction of the octalone (*2*). The resulting β-decalone (m.p. 111–112.5°; C, 84.57; H, 10.32) was then converted to the diacetic acid (*3*) (m.p. 191–192°; C, 72.80; H, 8.73) in 86% yield by ozonization of the 3-hydroxymethylene

(1) R. E. Ireland and P. W. Schiess, *Tetrahedron Letters*, No. 25, 37 (1960).
(2) G. Stork and J. W. Schulenberg, *J. Am. Chem. Soc.*, **78**, 250 (1956).
(3) W. E. Bachmann, G. I. Fujimoto, and L. B. Wick, *J. Am. Chem. Soc.*, **72**, 1995 (1950).
(4) W. S. Johnson, *Chem. and Ind.*, 167 (1956).
(5) R. Kierstead, unpublished results.

derivative. This diacid (*3*) was available in 81% over-all yield from the octalone (*2*) by the same operations but without purification of the intermediates.



When the diacid (*3*) was treated with polyphosphoric acid at 80° for 15 min. under a nitrogen atmosphere, there resulted a 75% yield of a tricyclic keto acid (m.p. 184–185°; C, 76.79; H, 8.59) which on hydrogenolysis over palladium-on-carbon furnished a 96% yield of *dl*-homodehydroabietic acid (*4*) (m.p. 173–174°; C, 80.30; H, 9.71), previously reported by Stork and Schulenberg.[2]

Degradation of the homoacid (*4*) to *dl*-dehydroabietic acid (*5*)[2] (m.p. 178.5–180°; C, 79.89; H, 9.40) was accomplished in 43% over-all yield through the sequence: *N*,*N*-dimethylamide (m.p. 78–79°; C, 80.70; H, 10.16; N, 4.20) to the corresponding *N*,*N*-dimethylamine (oil; C, 84.06; H, 11.28; N, 4.46); thence to the vinyl derivative (oil; C, 89.00; H, 10.57) by pyrolysis of the *N*-oxide; and finally permanganate–periodate oxidation.[6] Infrared spectral comparison of this *dl*-acid (*5*) with (+)-dehydroabietic acid as well as the *dl*-methyl ester with (+)-methyl dehydroabietate established the identity of the synthetic material and the naturally derived substances.

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(6) R. E. Lemieux and E. von Rudloff, *Canad. J. Chem.*, **33**, 1701 (1955).

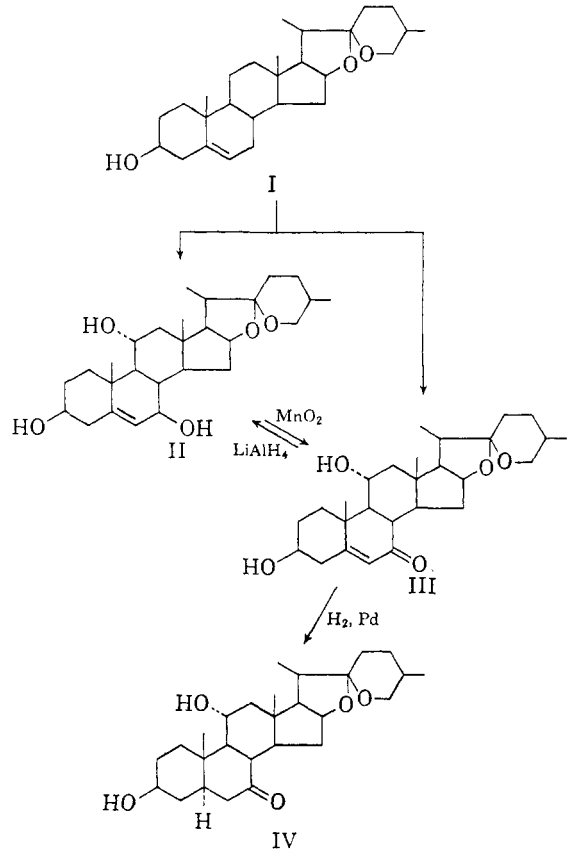
(7) Stauffer Chemical Company Fellow, 1961–1962.

Microbiological Transformation of Diosgenin

Sir:

We have recently reported the microbiological hydroxylation of the two amino analogs of the steroidal sapogenins, *i.e.*, of solasodine and tomatidine with *Helicostylum piriforme*[1] (A.T.C.C. 8992).

We wish now to report the successful microbiological transformation of diosgenin, a steroidal sapogenin, which has been reported[2] as not being readily amenable to hydroxylation. The incubation of diosgenin (I) with *H. piriforme* resulted in the formation of 7β,11α-dihydroxydiosgenin (II), m.p. 263–266°, $[\alpha]_D^{25}$ −47.0° (C_2H_5OH) (Found: C, 72.53; H, 9.75) (yield, 10–15%) and 11α-hydroxy-7-oxodiosgenin (III), m.p. 221–225°, $[\alpha]_D^{25}$ −137.1° (C_2H_5OH), $\lambda_{max}^{alc.}$ 239 mμ (log ϵ 4.07) (Found: C, 73.08: H, 8.78) (yield 5–10%).



The structures of II and III were deduced from the following facts. Oxidation with manganese dioxide in chloroform at room temperature readily converted substance II into an α,β-unsaturated carbonyl derivative which was found to be identical with product, III, obtained directly in the fermentation. Compound III in turn on catalytic reduction (palladium-charcoal, acetic acid) afforded the 5,6-dihydro derivative IV the properties of which were in agreement with an authentic specimen of 22-isoallospirostan-3α,11α-diol-7-one[3] (IV). Molecular rotation data (ΔM_D +223) together with the fact that lithium aluminum hy-

(1) Y. Sato and S. Hayakawa, *J. Org. Chem.*, **26**, 4181 (1961).

(2) R. F. Mininger, M. E. Wall, R. E. Dworshack and R. W. Jackson, *Arch. Biochem. and Biophys.*, **60**, 427 (1956).

(3) C. Djerassi, E. Baties, M. Velasco, and G. Rosenkranz, *J. Am. Chem. Soc.*, **74**, 1712 (1952). We thank Dr. Otto Halpern of Syntex, S. A. Mexico, for providing us with a specimen of the ketone.

dride reduction of III affords II in preponderant amounts leads us to ascribe the β-configuration to the 7-hydroxyl moiety. It has been shown[4] that the lithium aluminum hydride reduction of a 7-ketone leads predominantly to the formation of the 7β-isomer. In addition a small amount of a third component (m.p. 218–223°) which resembles compound IV in its spectral, paper, and gas chromatographic[5] behavior was isolated. However an unequivocal assignment of its structure warrants further study.

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(6) Visiting Scientist, National Institutes of Health.

A Disaccharide from Carboxyl-Reduced Heparin

Sir:

Acid hydrolysis of partially desulfated, acetylated, reduced, and partially de-*O*-acetylated heparin[1] was shown to yield D-glucose (I), 2-amino-2-deoxy-D-glucose (II) (hydrochloride), and a substance (III·HCl) $R_{glucose}$ 0.32[2]; the last is the subject of this Communication. Hydrolytic conditions for maximum yield of III were established by a plot of reducing power[3] against time, and confirmed paper chromatographically. The heparin derivative,[1] $[\alpha]^{25}D$ +52° (*c* 1, water) [*Anal.* Calcd. for $C_{24}H_{28}O_8(NHCOCH_3)_2(OCOCH_3)_{1.5}(OH)_{3.0}(OSO_2Na)_{0.5}$: C, 44.4; H, 5.8; N, 3.35; S, 1.91. Found: C, 43.96; H, 6.09; N, 3.50; S, 1.52] (2.45 g.) was refluxed for 4.5 hr. in 1.5*N* hydrochloric acid (245 ml.), neutralized (silver carbonate), concentrated, and resolved on nine sheets of Whatman 3MM paper to give I, II, III, and additional reducing zones $R_{glucose}$ 0.145 and 0.055. Substance III·HCl was crystallized from 1-propanol; yield 252 mg. (21%), m.p. 180–185° dec., $[\alpha]^{25}D$ + 100 (10 min.) → +81° (2.5 hr., equil., *c* 2.8, water), $\lambda^{KBr}_{max(\mu)}$ 3.1, 3.45, 6.15, 11.65; x-ray powder diffraction data[4]: 7.87 s (2,2), 4.30 vs (1), 3.15 m, 3.07 s (2,2), 2.87 s (3,3) 2.77 vw, 2.67m, 2.62 vw, 2.48 m, 2.19 m, 2.07 s (3,3), 1.9 m, 1.8 m. *Anal.* Calcd. for $C_{12}H_{24}ClNO_{10}$: N, 3.72. Found: N, 3.9. Hydrolysis of III·HCl with 2*N* hydrochloric acid for 2 hr. at 100° gave approximately equal quantities of I and II·HCl (papergram). Calculated as a disaccharide of I and II, III gave one half the color given by an equivalent of II in the Elson-Morgan test.[5] Under these conditions II 4-methyl ether gave 58% of the color given by II.[6] *N*-Acetylation[7] of III gave 76% of III *N*-acetyl derivative (IV), $R_{glucose}$ 0.46,[2] chromatographically homogeneous in three solvent systems; IV gave a positive Benedict test, a negative ninhydrin test, and crystallized from ethanol–ether; m.p. 144.5–146° (preliminary softening 138°), $[\alpha]^{19}D$ +85 (1 min.) → +39° (2.5 hr., equil., *c* 0.9, water); $\lambda^{KBr}_{max(\mu)}$ 2.97, 6.09, 6.46 (NHAc), 11.83; x-ray powder diffraction data[4]: 3.77 m, 2.76 vs, 2.29 s, 1.99 w, 1.91 m, 1.80 w, 1.48 m, 1.35 m. *Anal.* Calcd. for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.52; N, 3.65; mol. wt. 380. Found: C, 43.98; H, 6.27; N, 3.44; mol. wt.[8] 365. Substance IV gave no color in the Morgan-Elson[9] analytical determination.

Borohydride reduction[10] of IV (75 mg.), removal of boric acid (exhaustive methanol codistillation) and deionization gave 24.5 mg. (32.7%) of the alditol (V) derived from IV, as a chromatographically homogeneous nonreducing sirup, $R_{glucose}$ 0.30, $[\alpha]^{19}D$ +75° (*c* 0.45, water), which on hydrolysis with 2*N* hydrochloric acid for 2 hr. at 100° gave (papergram) a reducing component corresponding to I and a nonreducing component, $R_{glucose}$ 0.65, corresponding to 2-amino-2-deoxy-D-glucitol hydrochloride (VI). The hydrolyzate gave a negative test for free hexosamine in the Elson-Morgan[5] analytical determination, and a sample of crystalline VI isolated from the hydrolyzate gave an x-ray powder diffraction pattern identical to that of authentic VI.

These data would suggest the formulation of III·HCl as *O*-α-D-glucopyranosyl-(1 → 4)-2-amino-2-deoxy-α-D-glucopyranose hydrochloride. The data on V establish the sequence of units and give strong evidence for the configuration of the interglycosidic link. Since hexosamine forms the reducing moiety, the negative Morgan-Elson[9] reaction of IV indi-

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cates substitution at C-4 of the hexosamine.[11] This result is in accord with the recent finding[12] that C-3 of the hexosamine moiety of de-*N*-sulfated heparin is unsubstituted. Further studies for the unequivocal identification of III are in progress.

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