this instance the product did not arise *via* an esterification process.

We wish at this time to describe the 21-acetylation of 9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxy-pregn-4-ene-3,20-dione (I)[6] by a strain of *Trichoderma glaucum* (Lederle culture No. Z-696[7]), and to comment upon the substrate specificity of this novel steroidal transformation process.

Trichoderma glaucum was grown under conditions of aeration and agitation in a 20-liter glass fermenter containing 12 liters of a medium consisting of 2% molasses, 1% corn starch and 1% corn steep liquor. After a 48-hour incubation period, 3 g. of I in methanolic solution was introduced aseptically into the fermenter. The fermentation was permitted to proceed for an additional 53 hours, at which time the mash was extracted with ethyl acetate. The extract was concentrated *in vacuo* to a residue which was chromatographed on diatomaceous silica[8] employing the system water–methanol–dioxane–cyclohexane (1–1–4–5). A major fraction of ultraviolet-absorbing material was obtained at 1.8 holdback-volumes. From this fraction 2.1 g. of a crude crystalline product was obtained in 70% yield. Three recrystallizations from acetone–ether furnished analytically pure 21-acetoxy-9α-fluoro-11β-hydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (II); m.p. 239–240°; $[\alpha]^{25}\text{D}$ +136° (CH_3OH); (Found: C, 65.19; H, 7.34; F, 3.88). II was identical with the product obtained by chemical acetylation of I[9] on the basis of infrared and ultraviolet spectra, mixed melting point, and papergram mobility in three solvent systems. Hydrolysis of II with sodium carbonate in methanol provided analytically pure I, identical with an authentic sample on the basis of the above criteria.

While several species of *Trichoderma* have been reported to effect 17α-hydroxylation of steroids,[10] none of the strains that we have tested possessed this activity, though capacity for 21-esterification was relatively widespread among species of this genus.

Only a few steroids were acetylated by *T. glaucum* thereby indicating the high substrate specificity requirement of the enzyme.

Steroids which were 21-acetylated are listed: 9α-fluoro-16α-hydroxyhydrocortisone-16α,17α-acetone ketal (I), 9α-fluoro-16α-hydroxyprednisolone-16α,17α-acetone ketal, 16α-hydroxyhydrocortisone-16α,17α-acetone ketal and 16α,17α,21-trihydroxypregn-4-ene-3,20-dione-16α,17α-acetone ketal. No evidence was obtained for esterification of these compounds after fermentation with *T. glaucum:* 9α-fluoro-16α-hydroxyprednisolone-16α,17α-orthoformate, 9α-fluoro-16α-hydroxyhydrocortisone-16α,17α-borate, Na salt,[11] 9α-fluoro-16α-hydroxyhydrocortisone, 16α,21-dihydroxyprogesterone, 11-deoxycorticosterone and testosterone. Esterification was limited to C-21, and then only with substrates possessing a 16α,17α-isopropylidenedioxy group. A study of the mechanism of this reaction is in progress and will be the subject of a later communication.

Acknowledgment.—We wish to express our appreciation to P. Shu and co-workers for conducting the pilot plant fermentation, to L. Brancone and associates for microanalyses and to W. Fulmor and associates for spectral and optical rotation. data. Some of the steroids were kindly provided by R. Brownfield and L. L. Smith.

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[5] G. S. Fonken, H. C. Murray and L. M. Reineke, *J. Am. Chem. Soc.*, **82**, 5507 (1960).

[6] J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, *ibid.*, **80**, 2338 (1958).

[7] Culture Z-696 was isolated and identified by Dr. H. D. Tresner.

[8] Celite 545, Johns-Manville.

[9] We wish to thank S. Bernstein and R. H. Lenhard for a sample of chemically prepared II.

[10] (a) W. J. McAleer and E. L. Dulaney, *Arch. Biochem. and Biophys.*, **62**, 111 (1956). (b) British Patent 759,731, October 24, 1956.

[11] L. J. Leeson, J. A. Lowery, G. M. Sieger and S. Muller, *J. Pharm. Sci.*, **50**, 193 (1961).

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THE SYNTHESIS OF ABIETIC ACID FROM DEHYDROABIETIC ACID

Sir:

With the completion of the total synthesis of dehydroabietic acid (I) in its racemic form by Stork and Schulenberg,[1] the transformation of this aromatic resin acid into the more abundant dienoid resin acids, such as abietic acid (II),[2] became an important goal in the field of diterpene chemistry.[3] This transformation now has been achieved in good over-all yield by application of the Benkeser lithium-in-ethylamine reduction procedure[4] under carefully defined conditions.

Finely divided lithium (2.78 g., 0.40 gram atom) was added over a period of 15 min. to a rapidly stirred solution (sodium-dispersion motor) of 3.0 g. (0.010 mole) of dehydroabietic acid (I)[5] in redistilled anhydrous ethylamine (375 ml.) and *tert*-amyl alcohol (45.5 ml., 0.42 mole)[6] at the reflux temperature. When dissolution of the lithium was nearly complete (*ca.* 30 min.), and the mixture had turned ink blue, sufficient *tert*-amyl alcohol (2–3 ml.) was added to discharge the color. After distillation of the ethylamine, the acidic product (III, contaminated with an estimated 15–20%

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[1] G. Stork and J. W. Schulenberg, *J. Am. Chem. Soc.*, **78**, 250 (1956).

[2] Although abietic acid is commonly regarded as a "secondary" resin acid, it has been shown by G. C. Harris and T. F. Sanderson, *ibid.*, **70**, 334 (1948), to be present in mineral acid-isomerized rosin to the extent of at least 47%. *Cf.* G. C. Harris and T. F. Sanderson, *Org. Syntheses*, **32**, 1 (1952).

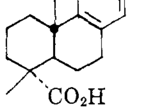
[3] *Cf.* J. A. Barltrop and A. C. Day, *J. Chem. Soc.*, 671 (1959).

[4] R. A. Benkeser, R. E. Robinson, D. M. Sauve and O. H. Thomas, *J. Am. Chem. Soc.*, **77**, 3230 (1955), and later papers. For a survey of recent advances in the subject, *cf.* R. H. Towe, MIT Organic Seminar Abstracts, First Semester, 1960–1961, pp. 155–163. See also R. A. Benkeser, M. L. Burrous and R. K. Agnihotri, Abstracts of Papers Presented at the 139th National A.C.S. Meeting, p. 14-O. We are grateful to Professor Benkeser for correspondence and discussions pertaining to material presented in this paper.

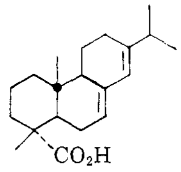
[5] We are indebted to Mr. T. F. Sanderson (Hercules Powder Co., Wilmington, Del.) for a generous supply of crystalline dehydroabietonitrile from which dehydroabietic acid, m.p. 171–172°, $[\alpha]\text{D}$ +64° (all rotations at concentrations of 1–2% in ethanol), was prepared *via* the recrystallized methyl ester, m.p. 62–62.5°, $[\alpha]\text{D}$ +62°.

[6] Any significant increase or decrease in the ratio of alcohol to lithium resulted, respectively, in incomplete reduction or over-reduction.

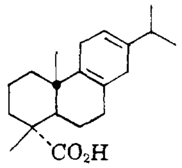
of IV) was isolated as the di-*n*-butylamine salt, which when recrystallized from acetone afforded 4.05 g. (94% yield) of glistening needles, m.p. 141–144°, $[\alpha]_D$ +31° (found for $C_{28}H_{49}NO_2$: C, 77.96; H, 11.34; N, 3.26). The m.p. and optical rotation were unchanged by further recrystallization. Treatment of the salt with acetic acid in dilute ethanol[2] yielded, after recrystallization from ethanol–water, 2.42 g. (80%) of $\Delta^{8,12}$-abietadienoic acid (III) (steroid-triterpene numbering) as fine plates, m.p. 160–162°, $[\alpha]_D$ +55°. The ultraviolet spectrum (only end-absorption in the low wave length region), the n.m.r. spectrum[7] in $CDCl_3$ (single vinyl proton, 4.55τ), and the mode of formation support the assignment of structure III.



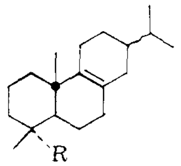
I



II



III



IV, R = CO_2H
V, R = CHO

When heated under reflux for 2 hr. in ethanol with concd. hydrochloric acid,[2] recrystallized III furnished, in 90% yield, abietic acid (II) of an estimated purity of 80–85%,[8] which was isolated as the diisoamylamine salt, m.p. 133–136°, $[\alpha]_D$ −47° (decreased to −61°[2] after three recrystallizations from acetone), or as the di-*n*-butylamine salt, m.p. 152–155°, $[\alpha]_D$ −33° (rotation not altered appreciably by further recrystallization; found for $C_{28}H_{49}NO_2$: C, 77.92; H, 11.30; N, 3.41). The physical properties of the regenerated, recrystallized[2] acid, including crystallization behavior,[2] m.p. and mixed m.p. (167–172°), optical rotation[2] ($[\alpha]_D$ −106°), infrared spectrum (CS_2 and KBr disk), ultraviolet absorption[2] [λ_{max} 235 (ε 21,500), 241.5 (23,000) and 250 mμ (15,500)], and n.m.r. spectrum[7] (two vinyl protons, 4.26 and 4.66τ), were identical with those of authentic abietic acid.[2,9,10]

When heated at 210° with potassium hydroxide in ethylene glycol, III was partially isomerized to palustric acid ($\Delta^{8,13}$-abietadienoic acid), λ_{max} 265–266 mμ (single vinyl proton in the n.m.r. spectrum,[7] 4.54τ). The further isomerization of palustric acid to abietic acid[11] and the conversion of abietic acid to neoabietic acid[2] and to dihydro- and tetrahydroabietic acids[12] have been reported previously.

Interestingly, in the absence of an alcohol, the lithium-in-ethylamine reduction of dehydroabietic acid gave a mixture of non-aromatic *aldehydic* products in about 80% yield. From this mixture, Δ^8-abietenal (V) was isolated in 20% yield by alumina chromatography of the 2,4-dinitrophenylhydrazone (m.p. 158–159°; found for $C_{26}H_{36}N_4O_4$: C, 66.69; H, 7.88; N, 11.85). The position of the double bond in V was verified by the n.m.r. spectrum[13] of the 2,4-DNP and by the method of Castells and Meakins[14] as applied to the corresponding carbinol.

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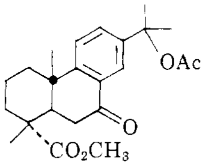
(7) This determination was obtained through the courtesy of Professor Ernest Wenkert at Iowa State University.

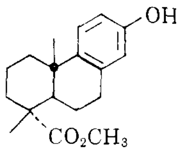
(8) Calculated on the basis of the observed extinction at 241.5 mμ.[2] The presumed impurity, Δ^8-abietenoic acid (IV), arising from a small amount of over-reduction of I, is reported to have m.p. 174–176°, $[\alpha]_D$ +104° [E. E. Fleck and S. Palkin, *J. Am. Chem. Soc.*, **59**, 1593 (1937)]. A 4:1 mixture of pure abietic acid and IV ($[\alpha]_D$ +85°), obtained by partial hydrogenation of III, showed spectral and optical rotational properties virtually identical with those of the acid isomerization product of III.

(9) Kindly supplied by Dr. Ray V. Lawrence, Naval Stores Research Laboratory, *Olustee, Fla.* We thank Dr. Lawrence for this courtesy and also for sending a supply of WW gum rosin from which additional pure abietic acid was isolated.[2]

(10) An alternative pathway from dehydroabietic acid to abietic acid also has been realized in our Laboratories: The acetoxy keto ester (i), m.p. 126–130°, $[\alpha]_D$ +5°, prepared by chromic acid oxidation of methyl dehydroabietate (T. F. Sanderson, U. S. Patent 2,750,368, issued June 12, 1956), was subjected to Wolff–Kishner reduction and hydrolysis. The resulting hydroxy acid, m.p. 186–187°, $[\alpha]_D$ +49° (84% yield), was esterified (diazomethane) and converted via the corresponding hydroperoxide (*cf.* T. F. Sanderson, U. S. Patent 2,750,367, issued June 12, 1956) to the phenolic ester (ii), m.p. 149.5–150°. O-Methylation, ester hydrolysis, Benkeser–Birch reduction,[4] esterification (diazomethane), and then reaction of the resulting keto ester, m.p. 127–128° [G. C. Harris and T. F. Sanderson, *J. Am. Chem. Soc.*, **70**, 339 (1948); *cf.* ref. 3], with isopropylmagnesium bromide in benzene-ether, followed by dehydration and hydrolysis, furnished abietic acid.



(i)



(ii)

Recently, we have learned that a similar sequence, based on the transformation of desisopropyldehydroabietic acid [E. Wenkert and J. W. Chamberlin, *J. Am. Chem. Soc.*, **81**, 688 (1959); *cf.* M. Ohta and L. Ohmari, *Pharm. Bull.* (*Tokyo*), **5**, 91, 96 (1957)] to the phenolic ester (ii), also has been carried out in the Iowa State Laboratories (private communication from Professor Wenkert, with permission to quote).

(11) V. M. Loeblich, D. E. Baldwin and R. V. Lawrence, *J. Am. Chem. Soc.*, **77**, 2823 (1955).

(12) *Cf.* J. L. Simonsen and D. H. R. Barton, "The Terpenes," Vol. III, Cambridge Univ. Press, New York, N. Y., 1952, pp. 407–418.

(13) We thank Dr. James N. Shoolery for this determination.

(14) J. Castells and G. D. Meakins, *Chemistry & Industry*, 248 (1956).

(15) The senior author gratefully acknowledges the inspiration and encouragement of Professors Gilbert Stork and D. H. R. Barton. We both thank the University of Kansas for a grant from the General Research Fund.

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LAWRENCE, KANSAS LEONARD R. WORDEN

RECEIVED APRIL 8, 1961

THE SYNTHESIS OF ORTHOAMIDES AND THEIR CONVERSION TO FORMAMIDINIUM SALTS

Sir:

We wish to report the synthesis of a series of orthoamides, I. These materials are the first examples of compounds having three basic nitrogens attached to a single carbon atom.[1] They are

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(1) Bredereck, *et al.*, *Ber.*, **92**, 329 (1959), and **93**, 1398 (1960), recently have reported the preparation of a series of triacylaminomethanes. The chemistry of these materials is quite different from that of the orthoamides.