for both isomers. In addition we have determined the pseudo first-order rate of oxygen exchange for both isomers to learn more about the excess racemization.

The three measured reactions: (a) loss of optical activity (eq. 1), (b) isomerization (eq. 2) and (c)

$$\textit{active}\text{-ROH} \xrightarrow{k_\alpha} \textit{inactive}\text{-ROH} \quad (1)$$

$$\textit{cis}\text{-ROH} \underset{k_{i\ trans}}{\overset{k_{i\ cis}}{\rightleftharpoons}} \textit{trans}\text{-ROH} \quad (2)$$

$$RO^{18}H \xrightarrow{k_{exc}} ROH \quad (3)$$

oxygen exchange (eq. 3), are cleanly pseudo first-order, *i.e.*, these processes are first-order in acid (the concentration of which remains constant) and alcohol. The kinetic behavior is consistent with the interpretation that these processes involve reversible protonation of the alcohols followed by first-order transformations of the conjugate acids of the alcohols.[1] If the three rates are compared at the same acid concentration the relative magnitudes of the pseudo first-order constants correspond to those of the first-order constants for transformations of the conjugate acids of the alcohols. The pseudo first-order constant for the stereospecific racemization (k_{rac}, eq. 4) is $k_\alpha - k_i$.

$$d\text{-I} \xrightarrow{k_{rac\ cis}} dl\text{-I} \quad (4a)$$

$$d\text{-II} \xrightarrow{k_{rac\ trans}} dl\text{-II} \quad (4b)$$

Pseudo first-order constants for reactions 1–4 at 30° in 35% aqueous acetone containing 0.095 *M* $HClO_4$ are given in Table I. Rate constants for isomerization, $k_{i\ cis}$ and $k_{i\ trans}$, were determined from the pseudo first-order rate of equilibration ($k_{i\ cis} + k_{i\ trans}$) and the equilibrium constant for equation 2; $K_{eq} = k_{i\ cis}/k_{i\ trans} = 1.22$. The same values for K_{eq} and ($k_{i\ cis} + k_{i\ trans}$) were obtained with both isomers. The isomerizations were followed by capillary gas chromatography which is superior to the method used in the earlier work (infrared analysis).[1] The rates of O^{18} exchange were determined using labeled I and II. In these experiments samples of alcohol were isolated periodically by gas chromatography without fractionation of the isomers and the O^{18} contents of the alcohol fractions were determined by a previously described method.[2] Rates of loss of optical activity were determined in the usual manner.[1]

TABLE I

PSEUDO FIRST-ORDER RATE CONSTANTS FOR LOSS OF OPTICAL ACTIVITY (k_α) ISOMERIZATION (k_i) RACEMIZATION (k_{rac}) AND OXYGEN EXCHANGE (k_{exc}) FOR I AND II IN 35% AQUEOUS ACETONE ([$HClO_4$] = 0.095) at 30.2°

Isomer[a]	I	II
$10\ k_\alpha$, hr.$^{-1}$	1.46[b]	3.64[b,c]
$10\ k_i$, hr.$^{-1}$	0.15 ± 0.02	0.19 ± 0.02
$10\ k_{rac}$, hr.$^{-1}$	1.31	3.45
$10\ k_{exc}$, hr.$^{-1}$	0.23 ± .03	1.5 ± .3

[a] Substrate concentration is 0.5 *M*. [b] Taken from reference 1 and corrected for acid concentration of 0.095 *M*. [c] This value was reconfirmed in the present work.

The data presented in Table I reveal a most interesting phenomenon. In the *cis* system, the rate of oxygen exchange (k_{exc}) is far smaller than that of loss of optical activity and in fact corresponds quite closely to that of isomerization. Since isomerization undoubtedly involves exchange, this means that the excess racemization ($k_\alpha/k_i = 10$) is almost completely intramolecular, *i.e.*, an SNi′ process. Such schemes have been proposed earlier[1,3] for acid-catalyzed rearrangements of allylic alcohols, however, this appears to be the first demonstration of an intramolecular allylic rearrangement of this charge type.

The *trans* system behaves in a completely different way. In this case k_{exc} is much larger than k_i and nearly half as large as k_α. Or to put it another way, there is substantial exchange associated with the stereospecific excess racemization. If racemization involved an SN2′ displacement, k_{exc} would equal k_i plus $^1/_2 k_{rac}$ or 1.9 hr.$^{-1}$. The value is nearly this large, which shows that in this case racemization is substantially an intermolecular or SN2′-type process with a small contribution from the intramolecular process.

The reason for the different behavior of the two isomers is not clear. It seems unlikely that the conjugate acids of the isomeric alcohols react by fundamentally different mechanisms. If the rearrangement involves a carbonium ion mechanism it is obvious that the geometric isomers give rise to different intermediates. However, this difference may be in the conformation of the symmetrical 4-methyl-2-cyclohexenyl carbonium ion or in the way that the ion is solvated.

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(2) H. L. Goering and M. Pombo, *J. Am. Chem. Soc.*, **82**, 2515 1960).

(3) W. G. Young, K. Nozaki and R. Warner, *ibid.*, **61**, 2564 (1939); E. A. Braude, *Ann. Rept. Chem. Soc.*, **47**, 114 (1949); *Quart. Rev.*, **4**, 404 (1950).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN HARLAN L. GOERING
MADISON, WISCONSIN ROY R. JOSEPHSON

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MICROBIOLOGICAL ESTERIFICATION OF STEROIDS

Sir:

While many types of reactions may occur in fermentation processes,[1] the microbiological transformation of steroids most frequently involves oxidative, reductive or hydrolytic reactions.[2] Acetylation of steroids by biological systems has been reported only recently. The isolation of 11-dehydrocorticosterone acetate from normal peripheral human plasma[3] implies the presence of a C_{21}-acetyl transferase in mammals. Testosterone acetate has been obtained by fermentation of androstenedione with a strain of *Saccharomyces fragilis*.[4] The same ester was isolated from the fermentation of progesterone with *Cladosporium resinae*,[5] but in

(1) L. L. Wallen, F. H. Stodola and R. W. Jackson, "Type Reactions in Fermentation Chemistry," ARS 71-13, May 1959 (U. S. Department of Agriculture).

(2) E. Vischer and A. Wettstein, "Advances in Enzymology," Vol. XX, Interscience Publishers, Inc., New York, N. Y., 1958, p. 237. Earlier reviews are cited in this reference.

(3) T. E. Weichselbaum and H. W. Margraf, *J. Clin. Endocrinol. and Metabolism*, **20**, 1341 (1960).

(4) J. S. McGuire, E. S. Maxwell and G. M. Tomkins, *Biochim. et Biophys. Acta*, **45**, 392 (1960).

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°; $[\alpha]^{25}D$ +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.

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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).

C. E. HOLMLUND
BIOCHEMICAL RESEARCH SECTION L. I. FELDMAN
LEDERLE LABORATORIES DIVISION N. E. RIGLER
AMERICAN CYANAMID COMPANY B. E. NIELSEN
PEARL RIVER, NEW YORK R. H. EVANS, JR.

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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%

(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°, $[\alpha]D$ +64° (all rotations at concentrations of 1–2% in ethanol), was prepared via the recrystallized methyl ester, m.p. 62–62.5°, $[\alpha]D$ +62°.

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