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The Structure of the Lactone of "Hydroxytetrahydroabietic Acid"[1]

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The diterpenoid lactone, m.p. 131°, $[\alpha]_D$ $-3°$, which is obtained from various dihydroabietic-type acids by mineral acid treatment, has been shown not to have the previously accepted structure, 13α-hydroxyabietan-15-oic lactone (I). It has been concluded that the correct structure, which results from a methyl group migration prior to lactonization, is 12α-hydroxy-13β-methyl-12-nor-11β,14α-abietan-15-oic lactone (II). Experimental proofs involve characterizations of several derivatives of the lactone and conversion of the lactone to 3-ethylretene by a series of unambiguous reactions. The mechanism of the methyl group migration and lactonization are discussed, and a 13β,14α-B/C ring fusion for the lactone has been assigned.

When commercial, partially hydrogenated rosin[2] is treated with a strong mineral acid, the most readily isolable compound, in yields as high as 35%, is a saturated $C_{20}H_{32}O_2$ diterpenoid lactone, m.p. 131°, $[\alpha]_D$ $-3°$.[3] This lactone is recognized as a product of the dihydroabietic-type acid[4] fraction of the hydrogenated rosin, since it also can be obtained in high yields from various pure dihydroabietic and dihydrolevopimaric acids.[5,6] Because the lactone is saturated and its formation has been depicted as a simple hydration of a double bond followed by lactonization, it has been described in the literature as the lactone of "hydroxytetrahydroabietic acid."[7] The structural implications of this name together with the evidence that the lactone bridge extends to a tertiary position led to the assignment of structure I several years ago.[6] More recently Barton[8] has questioned this assignment by pointing out that I is not sterically possible in view of the evidence for a *trans* A/B ring fusion in the abietic acid skeleton. As an alternative Barton suggested structure II on the premise that formation of the lactone from a dihydroabietic acid involves a carbonium ion process in which the angular methyl group migrates from C-12 to C-13.

In this present work three preliminary observations were made which support the correctness of this latter structure II.

First, an examination of the lactone's infrared spectrum showed a carbonyl absorption maximum (5.65 μ) that is somewhat shifted from the absorption maxima (*ca.* 5.75 μ) of open chain ester and δ-lactone carbonyls. Such a shift is indicative of the γ-lactone in II, for carbonyl maxima of γ-lactones are normally shifted to this extent.[9] The conclusiveness of this evidence is of course subject to some qualification, since the possibility of the lactone's complex and rigid structure effecting a similar increase in C=O vibrational stretching frequency[9b] cannot be eliminated.

Second, a lithium aluminum hydride reduction of the lactone gave a diol III, which on subsequent treatment with concentrated sulfuric acid dehydrated to a $C_{20}H_{32}$ conjugated diene product IV as evidenced by its ultraviolet spectrum, $\lambda_{\max}^{\text{alc}}$ 244 mμ (α, 37).[10] Ozonolysis of this diene product gave acetaldehyde. These results are more reasonably interpreted with the structures assigned than with the corresponding structures that would result from I.

Third, the unsaturated acids, m.p. 147–148° and m.p. 186–187° (Va and VI), which are obtained from the abnormal reaction of methylmagnesium iodide with the lactone,[11] were found to decarboxylate with the same facility that is normally observed with β,γ-unsaturated acids that are capable of a recently proposed cyclic transition complex between the carboxyl group and the double bond.[12] The decarboxylation yields of these acids were 96 and 51%, the latter being obtained from the lower melting acid Va which presumably decarboxylated by prior thermal isomerization to the higher melting acid VI. The alternative structures for these acids if derived from I cannot decarboxylate *via* this cyclic mechanism. Dehydroabietic acid (VII) and dihydrodextropimaric acid (VIII) which have similar mechanistic restrictions are completely stable under the same pyrolytic conditions.

Evidence of a more unequivocal nature was obtained from the following sequence of reactions. The acid Va was converted to its methyl ester Vb and then oxidized with molecular oxygen. This oxidation, which was carried out at 90° and employed a cobalt naphthenate catalyst, was greatly facilitated by the oily consistency of Vb. The desired oxidation product, methyl 3-oxo-13β-methyl-12-nor-11β,14α-abieta-4-en-15-ate (IXa),[13] was obtained in an over-all yield of 22% after isolation and purification by use of Girard reagent and chromatography. Saponification of IXa followed by careful acidification gave the corresponding acid IXb, which on crystallization from methanol lactonized

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(1) Presented before the Division of Organic Chemistry, 125th Meeting, Am. Chem. Soc., Kansas City, Mo., March, 1954.

(2) This product is derived from the Pinus palustris and similar conifers, and is produced by Hercules Powder Company under the trade name of "Staybelite Resin."

(3) E. E. Fleck and S. Palkin, THIS JOURNAL, **61**, 1230 (1939); R. F. B. Cox (assigned to Hercules Powder Company), U. S. Patent 2,355,-782.

(4) Monoölefinic resin acids possessing the carbon skeleton of abietic acid.

(5) L. Ruzicka and J. Meyer, *Helv. Chim. Acta*, **5**, 315 (1922); L. Ruzicka, H. Waldmann, P. J. Meier and H. Hosli, *ibid.*, **16**, 169 (1933).

(6) E. E. Fleck and S. Palkin, THIS JOURNAL, **61**, 3197 (1939).

(7) T. Hasselstrom and J. D. McPherson, *ibid.*, **60**, 2340 (1938).

(8) D. H. R. Barton, *Chemistry and Industry*, 638 (1948).

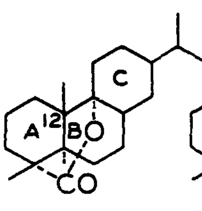
(9) (a) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1076 (1949); (b) J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 881 (1951).

(10) This diene product is recognized as a mixture of conjugated dienes of which IV is a representative structure.

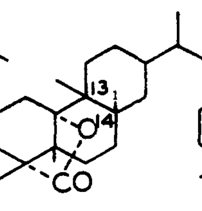
(11) R. F. B. Cox, THIS JOURNAL, **66**, 865 (1944).

(12) R. T. Arnold, O. C. Elmer and R. M. Dodson, *ibid.*, **72**, 4359 (1950).

(13) The diterpenoid nomenclature in this paper follows the recent proposal of W. Klyne, *J. Chem. Soc.*, 3072 (1953), which employs the general principles of steroid nomenclature and is based on the parent hydrocarbon abietane, the natural congener of 1,1-dimethyl-4aβ-methyl-7ξ-isopropyl-4bα,8aβ,10aα-perhydrophenanthrene.



I



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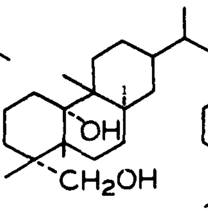

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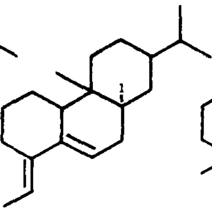

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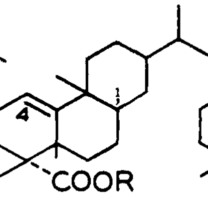
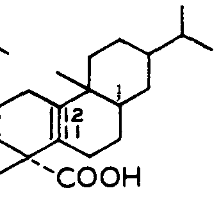
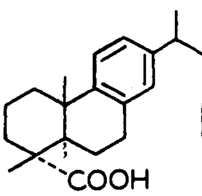


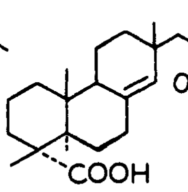
V a, R=H
b, R=CH_3



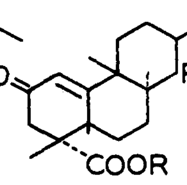
VI



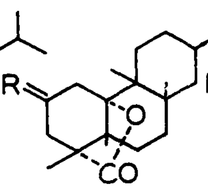
VII



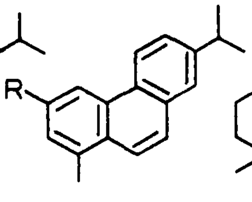
VIII



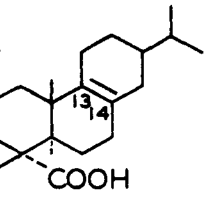
IX a, R=CH_3
b, R=H



X a, R=O
b, R=<S S]



XI a, R=H
b, R=C_2H_5



XII

to 12α-hydroxy-3-oxo-13β-methyl-12-nor-11β,14α-abietan-15-oic lactone (Xa). This ketone lactone, which also exhibited the shifted carbonyl maximum at 5.65 μ, was then treated with an excess of methylmagnesium iodide and the total product in turn dehydrogenated over palladium-on-carbon to a mixture of retene (XIa) and 3-ethylretene (XIb). The identity of the 3-ethylretene and its picrate was established by mixture melting point methods and infrared and X-ray diffraction comparisons with authentic samples of 3-ethylretene[14] and 3-ethylretene picrate.

The fact that 3-ethylretene was obtained from this reaction sequence substantiates the assigned position of the keto group in IXa and Xa. The α,β-unsaturation of IXa as evidenced by its ultraviolet spectrum, $\lambda_{\max}^{\text{alc}}$ 242 mμ (ε, 15,000), locates the double bond in Va(b) and IXa(b) and eliminates C-12 as a site of attachment for the angular methyl group in these compounds. To exclude the possibility of carbon skeletal rearrangements occurring during the over-all conversion of the original lactone to Xa, the ethylene dithioketal derivative (Xb) of Xa was reduced back to the original lactone by a Raney nickel desulfurization.

Although the foregoing experimental observations eliminate I as the structure of the lactone, they fall short of completely proving the alternative structure II, since the new position of the migrated angular methyl group remains undetermined. The lactone, however, is obtainable from a number of dihydroabietic-type acids[5,6]; consequently, a common intermediate must be involved before these various acids can undergo methyl migration and lactonization. The only intermediate that would not require extensive and unlikely double bond shifting and still provide an angular position and a double bond structure stable to strong acid is XII (or some protonated form of XII). Two angular positions, however, are available to the migrating methyl group in this intermediate, C-13 and C-14. Migration to the latter position seems improbable because of the considerable atomic movement that would be involved. Migration to C-13, on the other hand, could occur with relatively no movement of the methyl group. It therefore seems reasonable to conclude C-13 is the only position available to the migrating methyl group, and II is the correct structure of the lactone.

The B/C ring fusion in II is governed by the mode of addition of proton and the migrating methyl group to the double bond in XII. Existing knowledge on the stereochemistry of ionic additions to double bonds,[15] would seem to require this addition to be *trans*. Because of this and because steric restrictions limit the approach of the migrating methyl group to the β-side of C-13, an α-configuration has been assigned to the 14-hydrogen in II.

The formation of the lactone from a dihydroabietic-type acid must also involve the $\Delta^{11(12)}$-acid VI as an intermediate, since an epimerization at C-11 is required in order to provide the *trans* A/B ring fusion that is necessary for the strain free lactone structure in II.[8,16] Although the energy that is required for the actual methyl group migration could result from the stabilization that is gained by the lactonization of this intermediate $\Delta^{11(12)}$-acid, an alternative and more directly associated energy source can be found in the repulsive interactions that exist between the 1β- and 12β-methyl groups before migration. These methyl groups oc-

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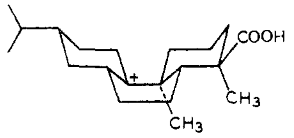
[14] The authentic sample of 3-ethylretene was prepared from retene by methods described in the literature (see experimental section). Although the structure of this compound was controversial for some time, it has been definitely proved by the work of W. P. Campbell and D. Todd, THIS JOURNAL, **62**, 1287 (1940).

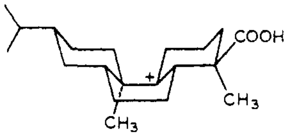
[15] M. J. S. Dewar, "Electronic Theory of Organic Chemistry," Oxford University Press, London, 1949, pp. 141–144.

[16] Further support for a *trans* A/B ring fusion can be found in the conversion of the free acid VI to the lactone by the action of alcoholic hydrochloric acid (see ref. 11). Such a reaction should generate the most stable structure possible and proceed by a *trans* addition of proton and carboxylate to the 11,12-double bond.

cupy polar positions[17] of extreme proximity on the same side of the reacting ion. Assuming other intramolecular steric interactions are equivalent as in the two ionic stages, XIII and XIV, the energy released by the separation of these methyl groups can be estimated from the strain energy values developed by Pitzer, *et al.*[18] In essence this involves obtaining the difference between the energy differences of the tautomeric forms of *cis*-1,3-dimethylcyclohexane and *trans*-1,4-dimethylcyclohexane.[18b] The result, 1.8 kcal./mole, is in the order of magnitude of the heats of isomerization observed for somewhat simpler systems,[19] and in the absence of any significant unfavorable entropy effects would seem to be in itself a sufficient cause to bring about the migration of the angular methyl group.



XIII



XIV

Experimental[20]

13β-Methyl-12-nor-11β,14α-abietan-12α,15-diol (III).—A 10.0-g. (0.034 mole) sample of the lactone, m.p. 131°, [α]D −3°, dissolved in 500 cc. of benzene was added to a stirring mixture of 5.60 g. (0.13 mole) of lithium aluminum hydride and 500 cc. of dry ether. After continued stirring at room temperature for 12 hours, the reduction mixture was treated with a few cc. of water and then with 100 cc. of 20% sulfuric acid. The organic phase that resulted was separated, washed and dried. Evaporation gave 9.14 g. of crystalline product (90% yield), which upon slow melting exhibited two melting points, 137.5–138.5° and 149–150°. Several recrystallizations from isoöctane failed to change these melting points; $[\alpha]^{25}D$ +28° ($CHCl_3$).

Anal. Calcd. for $C_{20}H_{36}O_2$: C, 77.86; H, 11.76. Found: C, 77.95; H, 11.77.

Acetylation of this diol with acetic anhydride and a little pyridine gave its monoacetate, which was crystallized from methanol–water; m.p. 68–69°, $[\alpha]^{25}D$ +15° ($CHCl_3$).

Anal. Calcd. for $C_{22}H_{38}O_3$: C, 75.38; H, 10.93. Found: C, 75.24; H, 11.00.

Benzoylation of this diol with benzoyl chloride and a little pyridine gave its monobenzoate, which was also crystallized from methanol–water; m.p. 104–105.5°, $[\alpha]^{25}D$ +7.0° ($CHCl_3$).

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found: C, 78.57; H, 9.86.

Dehydration of this diol with concentrated sulfuric acid at room temperature for six hours gave a diene hydrocarbon product in 71% yield after purification by chromatography and distillation; b.p. 140–142° (0.3 mm.), λ_{max}^{alc} 244 mμ (α, 37).

Anal. Calcd. for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 88.25; H, 12.08.

Ozonolysis of this diene product in ethyl chloride at −70° and decomposition of the ozonide mixture with zinc and acetic acid gave acetaldehyde which was isolated as its 2,4-dinitrophenylhydrazone (in a 6% yield and identified by X-ray diffraction methods) by blowing the volatile decomposition gases with nitrogen through an aqueous saturated 2,4-dinitrophenylhydrazine hydrochloride solution.

Decarboxylation of Va and VI.—A 2.062-g. sample of the $\Delta^{11(12)}$-acid (VI), m.p. 185–186°,[11] was heated at 240–250° for four hours. The evolved gas was swept up in a nitrogen stream and passed through a standard microanalytical carbon dioxide absorption train; yield of carbon dioxide, 286 mg. (96%). A similar experiment with 1.733 g. of the Δ^4-acid (Va), m.p. 147–148°,[11] yielded 128 mg. (51%) of carbon dioxide. The decarboxylation residue from $\Delta^{11(12)}$-acid was collected and distilled to give 1.20 g. of pure decarboxylated hydrocarbon, b.p. 123–124° (0.07mm.).

Anal. Calcd. for $C_{19}H_{32}$: C, 87.61; H, 12.39. Found: C, 87.87; H, 12.43.

Two-gram samples of dehydroabietic acid (m.p. 171–172°, [α]D +61°) and dihydrodextropimaric acid (m.p. 241–243°, [α]D +19.2°)[21] when heated under the same conditions failed to evolve any carbon dioxide and rendered the starting acids unchanged.

Methyl 3-Oxo-13β-methyl-12-nor-11β,14α-abieta-4-en-15-ate (IXa).—A 60-g. sample of the Δ^4-acid (Va), m.p. 146–146.5°,[11] was stirred with a refluxing mixture of potassium carbonate (60 g.), methyl iodide (42 g.) and dry acetone (300 cc.) for 24 hours. After removal of the volatile, water-soluble and acidic materials from this reaction mixture, 61 g. (97% yield) of the oily Vb was isolated. Sixty grams (0.19 mole) of this ester together with 20 drops of a cobalt naphthenate catalyst solution (6% cobalt) were then stirred in an atmosphere of oxygen at 90° for 25 hours. At various intervals (two to five hours) during this reaction period the oxygen atmosphere was replaced with fresh oxygen. By means of a gas buret attached to the reaction vessel, it was determined that a total of 7.36 g. (0.23 mole) of oxygen had been absorbed by the molten reaction mixture during its oxidation.

The total oxidate (63.0 g.) was then subjected to a Girard separation which gave 20.7 g. of ketonic material (λ_{max}^{alc} 242 mμ (α, 33)) and 41.0 g. of "non-ketonic" material (λ_{max}^{alc} 246 mμ (α 12)). Chromatographic purification of the ketonic fraction through a 1″ × 28″ basic alumina column eluting with hexane–benzene (1:1) gave 14.9 g. of crystalline product (λ_{max}^{alc} 242 mμ (α, 43)). Recrystallization of this from methanol at −5° yielded 13.4 g. of pure IXa; m.p. 59.5–60.5°; $[\alpha]^{25}D$ +235° ($CHCl_3$); λ_{max}^{alc} 242 mμ (ε, 15,000); $\lambda_{max}^{c=0}$ 5.75 μ, 5.94 μ (Nujol).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.82; H, 9.67.

A 2,4-dinitrophenylhydrazone derivative was prepared, m.p. 181–182°.

Anal. Calcd. for $C_{27}H_{36}N_4O_6$: N, 10.93. Found: N, 10.86.

12α-Hydroxy-3-oxo-13β-methyl-12-nor-11β,14α-abietan-15-oic Lactone (Xa).—A mixture consisting of 2.69 g. of IXa, 2.69 g. of potassium hydroxide, 20 cc. of diethylene glycol and 1 cc. of water was refluxed for 1.5 hours and then poured into 100 cc. of water and neutralized carefully with dilute hydrochloric acid. The product was extracted with ether and, after washing and drying, evaporation of this extract yielded 2.41 g. of an oil which crystallized upon standing. Recrystallization from methanol gave 1.87 g. (73% yield); m.p. 168–169°. Further recrystallization raised this melting point to 172–173°; $[\alpha]^{25}D$ −16° ($CHCl_3$), $\lambda_{max}^{c=0}$ 5.65 μ, 5.78 μ (CS_2).

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.20; H, 9.61.

The 2,4-dinitrophenylhydrazone of this compound melted at 182–184°.

Anal. Calcd. for $C_{26}H_{34}N_4O_6$: N, 11.24. Found: N, 11.16.

3-Ethylretene (XIb). A. From Retene.—A 17-g. sample of retene (Eastman Kodak) was converted to **3-acetylretene**

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[17] D. H. R. Barton, *Experientia*, **8**, 319 (1950).

[18] (a) K. S. Pitzer, *J. Chem. Phys.*, **8**, 711 (1940); (b) C. W. Beckett, K. S. Pitzer and R. Spitzer, THIS JOURNAL, **69**, 2488 (1947).

[19] G. Egloff, G. Hulla and V. I. Komarewsky, "Isomerization of Pure Hydrocarbons," Reinhold Publ. Corp., New York, N. Y., 1942, p. 35; A. Farkas, "Physical Chemistry of the Hydrocarbons," Academic Press, Inc., New York, N. Y., 1950, p. 383.

[20] All melting points are uncorrected. Ultraviolet absorption coefficients are α (specific) and ε (molar). Microanalyses by V. A. Aluise and N. A. Walker; ultraviolet spectra by E. V. Cook; infrared spectra by B. F. Brown; X-ray diffraction patterns by W. E. Fox, all of the Experiment Station, Hercules Powder Company. All rotations were observed in a 1-dm. polarimeter tube at 1% concentrations in the solvents indicated.

[21] T. Hasselstrom and B. L. Hampton, THIS JOURNAL, **61**, 967 (1939).

by a Friedel–Crafts reaction with acetyl chloride according to the procedure of Campbell and Todd[14]; yield 11.7 g. (57%); m.p. 96–97° (lit. m.p. 99.5–100°)[14] (Calcd. for $C_{20}H_{20}O$: C, 86.92; H, 7.29. Found: C, 86.63; H, 7.50); picrate derivative, m.p. 144.5–145° (lit. m.p. 142–143°).[22] A 1.20-g. sample of **3-acetylretene semicarbazone** was prepared, m.p. 246–247.5° (lit. m.p. 248.5–249.5°)[22] (Calcd. for $C_{21}H_{23}N_3O$: C, 75.65; H, 6.95; N, 12.60. Found: C, 75.32; H, 6.78; N, 12.68), and then reduced by a Wolff-Kishner reaction according to Bogert and Hasselstrom's direction[22] to give 0.72 g. (76% yield) of product. This was converted to **3-ethylretene picrate** (960 mg.), m.p. 150.5–151° (lit. m.p. 148–149°)[22] half of which was then chromatographed through an alumina column with benzene to give **3-ethylretene** (240 mg.), m.p. 51.0–52.0° (lit. m.p. 54–55°).[22]

Anal. Calcd. for $C_{20}H_{22}$: C, 91.55; H, 8.45. Found: C, 90.88; H, 8.93.

The trinitrobenzene derivative of 3-ethylretene was also prepared; m.p. 165–166°.

B. **From 12α-Hydroxy-3-oxo-13β-methyl-12-nor-11β,14α-abietan-15-oic Lactone (Xa).**—A solution consisting of 3.18 g. (0.01 mole) of Xa and 100 cc. of benzene was allowed to react at room temperature for 24 hours with a stirring Grignard solution which was made from 0.97 g. (0.04 mole) of magnesium, 10.2 g. (0.04 mole) of ethyl iodide and 100 cc. of ether. After the Grignard reaction mixture was decomposed with a 25% ammonium chloride solution (200 cc.), the organic layer was separated, washed with water and dried; evaporation yielded 3.45 g. of an oil. This oil was then mixed with an equal amount of 5% palladium-on-carbon and heated in a Heymann dehydrogenation apparatus[23] with a stream of nitrogen flowing over the reacting mixture for eight hours at 320–330°. An ether extraction of the dehydrogenated material yielded after evaporation 1.89 g. of an oil which slowly crystallized upon standing. A chromatographic separation of this product through a silica gel column with petroleum ether gave 0.20 g. of a non-fluorescent (to ultraviolet light) oily fraction and 1.12 g. of a fluorescent crystalline fraction. This latter material was then fractionally recrystallized from saturated picric acid ethanol solutions to yield 210 mg. of retene picrate[24] and 240 mg. of **3-ethylretene picrate**, m.p. 150.5–151°. Half of this latter picrate was chromatographed through an alumina column with benzene to give **3-ethylretene** (56 mg.), m.p. 51–52°. The trinitrobenzene derivative of this 3-ethylretene was also prepared; m.p. 161–162°.

The infrared spectra of the 3-ethylretenes obtained in A and B were indistinguishable. An X-ray diffraction pattern comparison of the 3-ethylretene picrates obtained in A and B proved their identicalness. Melting points of mixtures of the 3-ethylretene picrates, of the 3-ethylretenes, and of the trinitrobenzene derivatives obtained in A and in B were not depressed below their individual melting points.

Reduction of Xa to the Lactone.—A solution consisting of 200 mg. of Xa and 10 cc. of glacial acetic acid was treated with 1.0 cc. of boron trifluoride etherate and 0.5 cc. of ethanedithiol. The ethylene dithioketal product Xb precipitated almost immediately, and after a few minutes was filtered off (240 mg.) and recrystallized twice from ethanol; yield 190 mg., m.p. 289–291°, $\lambda_{\max}^{C=O}$ 5.62 μ (Nujol).

Anal. Calcd. for $C_{22}H_{34}O_2S_2$: C, 66.95; H, 8.68. Found: C, 67.04; H, 8.71.

Desulfurization was accomplished by refluxing 100 mg. of this dithioketal with 4 g. of freshly prepared Raney nickel for five hours in ethanol. Removal of catalyst and evaporation of solvent yielded 80 mg. of crystalline material, which exhibited the characteristic X-ray diffraction pattern of the original lactone and showed no depression in m.p. when mixed with an authentic sample of the original lactone.

WILMINGTON, DELAWARE

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[22] M. T. Bogert and T. Hasselstrom, THIS JOURNAL, **53**, 3462 (1931).

[23] L. F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., D. C. Heath and Co., New York, N. Y., 1951, p. 461.

[24] Identified by X-ray diffraction comparison with an authentic sample.

[CONTRIBUTION FROM THE EASTERN UTILIZATION RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Steroidal Sapogenins. XVI. Hydrolysis of 5α-22a-Spirostane Glycosides by Fungal Saponases[1,2]

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Certain microörganisms, especially those of the genera *Aspergillus* and *Penicillium*, when grown in a medium containing steroidal saponins with 5α,22a-spirostane aglycones adaptatively produce enzymes which cleave the saponins to sapogenins. The sapogenins thus obtained are identical with those secured as a result of cleavage of saponases from higher plants or by hydrochloric acid.

In a preceding paper[3] we reported that saponins with 5α,22a-spirostane aglycones can be split by saponases from higher plants into the component steroids and sugars. The present communication deals with the hydrolysis of similar steroidal glycosides by fungal saponases.

Stoll and co-workers[4] have shown that enzyme preparations from numerous fungi can cleave the carbohydrate–steroid linkage of certain cardiac glycosides. Similar enzyme preparations had no effect on 5α,22a-spirostane glycosides. We noted, however, that a purified saponin substrate contaminated by an actively growing mold was cleaved to the steroidal sapogenin. As a result of this discovery a large number of fungi were screened for their ability to grow in and to hydrolyze steroidal saponin substrates. The data are too voluminous to tabulate, but pertinent experimental results are presented in this paper.

Of the various fungi tested, several, but not all, species of *Aspergillus* and *Penicillium* gave the best results. In some instances, moreover, different strains of the same species gave opposite results, one strain being active and the other inactive. Species of these genera and a few others which hydrolyzed significant amounts of saponin are presented in Table I. A number of genera, species of which have failed to hydrolyze 5α,22a-spirostane glycosides, are as follows: *Absidia, Botrytis, Chaectomium, Circinella, Cunninghamella, Gliocladium, Helicostylum, Monilia, Mucor, Myrothecium, Oid-*

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[1] Paper XV, M. E. Wall, *et al.*, AIC 367, in press.

[2] Presented in part at the Philadelphia Meeting-in-Miniature of the American Chemical Society, January 29, 1953. Article not copyrighted.

[3] M. M. Krider and M. E. Wall, THIS JOURNAL, **76**, 2938 (1954).

[4] A. Stoll, J. Renz and A. Brack, *Helv. Chim. Acta*, **34**, 397 (1951).