Hydroxylation of Some Dehydroabietanes with *Corticium sasakii*

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Received April 17, 1968

Incubation of methyl dehydroabietate (**1**) with *C. sasakii* gives methyl 3β-hydroxydehydroabietate (**2**) and methyl 3β,7β-dihydroxydehydroabietate (**3**). Incubation of **2** or methyl 7β-hydroxydehydroabietate (**4**) also gives diol **3**. Incubation of methyl 7-oxodehydroabietate (**12**) gives methyl 3β-hydroxy-7-oxodehydroabietate (**10**) as an intermediate to methyl 3β,6β-dihydroxy-7-oxodehydroabietate (**15**). Similarly, incubation of methyl 7,18-dioxodehydroabietate gives methyl 3β,6β-dihydroxy-7,18-dioxodehydroabietate. Hydroxylation appears to occur first in the C-3β position, then in the C-6β or C-7β position of the dehydroabietanes. Oxygenation of dehydroabietanes at C-3, C-6, or C-7 by this fungal oxidase(s) is analogous to the positions of oxygenation of dehydroabietanes obtained from *Juniperus* trees.

Hydroxylation and oxidation of steroids and alkaloids with fungi is well known; however, diterpenes have not received similar attention[2] even though numerous highly oxygenated diterpenoids, *i.e.*, the gibberellins, are produced by fungi. The abietanes are one of the most abundant diterpenoid families found in higher plants.

Incubation[3] of methyl dehydroabietate (**1**) with *C. sasakii* (Lilly C-616) for 96 hr gives alcohol **2** and diol **3** (Scheme I). The assignment of the hydroxyl in **2** to C-3 is based upon the following argument. The nmr spectrum of **2** shows the proton attached to the carbon bearing the hydroxyl as a multiplet at 4.03 ppm indicative of an axial proton next to a methylene. The spectrum of **2** also shows the narrow envelope of the A-ring methylene protons in **1** split into a complex multiplet overlapping the 5α and C-6 methylene multiplet. The C-17 methyl signal in the nmr of alcohol **2** is not shifted, and in **5**, the corresponding acetate, it is shifted only 0.06 ppm from the position of the C-17 methyl signal in the nmr spectrum of methyl dehydroabietate. See Table I for nmr data. Oxidation of **2** gives ketone **6** whose infrared spectrum shows a nonconjugated carbonyl. The position of the C-17 methyl group in the nmr spectrum of **6** is 0.24 ppm downfield from that of methyl dehydroabietate. Based upon steroid models,[4,5] such a downfield shift of the C-17 methyl group could only be explained if the carbonyl is located at C-1 or C-3. This conclusion is supported, as deuteration[6] of ketone **6** gives compound **7** whose mass spectrum and nmr spectrum indicate the gain of only two deuteriums. The ketone is assigned to C-3 because of the near identity of the nmr spectrum[7] of **6** with the nmr spectrum of synthetic methyl norisopropyl-3-oxodehydroabietate. If keto ester **6** possess a carbonyl at C-3, then the corresponding keto acid should readily decarboxylate to give ketone **8**. Treatment of **6** with base and then with strong acid gave a compound to which we assign structure **8** on the basis of its nmr and mass spectral data. The nmr spectrum of **8** shows a doublet for the C-4α methyl at δ 1.26 (J = 7 cps).

Assignment of a β configuration to the C-3 hydroxyl in **2** is based upon chemical and nmr evidence. Reduction of ketone **6** with sodium trimethoxyborohydride gives only alcohol **2**. Even less bulky hydrides have been used[8] to reduce triterpenoid 3-oxo-4-*gem*-dimethyl systems to give the corresponding 3β-hydroxy compound. It will be shown below that the C-3 hydroxyl group in compound **19** is identical with the hydroxyl group in **2**. Analysis of the nmr spectrum of compound **19** gives A-ring proton coupling constants which would be expected only if the C-3 hydroxyl were in the equatorial position. The accuracy of these spectral assignments was verified by their use in a computer calculation of a theoretical signal for the 3α proton of compound **19**. Using the chemical shifts and coupling constants shown below, the FREQINT IV[9] program gives a spectrum of 210 lines. The contour of the plotted spectrum closely matches the signals of the A-ring protons in the observed spectrum of **19**, especially in the width and multiplicity of the C-3α proton (Table II). The downfield shift of 0.29 ppm for the C-15 methyl in the nmr spectrum of **2** obtained in pyridine is also indicative of the β configuration[10] of the hydroxyl in **2**. The C-15 methyl shifts downfield only 0.05 ppm in the pyridine nmr spectrum of **1**.

The nmr spectra of diol **3** and of the corresponding diacetate **9** both show two protons attached to carbons bearing hydroxyl (or acetoxyl) groups. The chemical shift and appearance of the lower field proton in both spectra are identical with the C-7α proton of methyl 7β-hydroxydehydroabietate and methyl 7β-acetoxydehydroabietate, respectively. The assignment of this hydroxyl of **3** to the C-7β position is supported by the identical shifts of the aromatic protons in the nmr spectrum of **3** and methyl 7β-hydroxydehydroabietate, relative to the aromatic proton signals of methyl dehydroabietate. The higher field signal of a proton attached to a carbon bearing a hydroxyl (or acetoxyl)

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(2) Biellmann, *et al.* [*Chem. Commun.*, 168 (1968)], have recently reported the isolation of the bacterium *Flavobacterium resinovorum* from the soil of a *Pinus maritima* forest which is able to use the nonvolatile portion of eleoresin from pine trees as the sole carbon source for its growth. Incubation of dehydroabietic acid with this organism gave them ketone **8**. They propose that **8** arises *via* enzymatic hydroxylation of dehydroabietic acid at C-3 and oxidation of the resulting alcohol to a ketone, which then undergoes decarboxylation.

(3) A preliminary account of this work has been published: *Chem. Commun.*, 681 (1968).

(4) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 19.

(5) See E. Wenkert, *et al.*, *J. Org. Chem.*, **30**, 713 (1965), for a comprehensive study of the nmr spectra of tricyclic diterpenic substances and a discussion of the conformation of 3-oxodehydroabietates.

(6) A convenient and efficient procedure was used to deuterate ketones **6**, **10**, **11**, and **12**. Approximately 20 mg of the ketone has dissolved in $CDCl_3$ in an nmr tube and 100 mg of 38% DCl in D_2O added. The tube was shaken at room temperature and nmr spectra were periodically obtained until deuteration appeared complete. Shaking for 2 hr was required for the above ketones. After removal of the DCl solution, the $CDCl_3$ solution in the nmr tube was washed with H_2O, then concentrated under vacuum. Mass spectral analysis of the resulting crystalline deuterated product showed greater than 90% exchange of all enolizable protons.

(7) Kindly furnished by Professor Ernest Wenkert, Indiana University, Bloomington, Ind.

(8) W. Lawrie, J. McLean, and J. Watson, *J. Chem. Soc.*, 1776 (1967).

(9) Obtained from A. A. Bothner-By, Mellon Institute, Pittsburgh, Pa.

(10) P. V. Demarco, *et al.*, submitted for publication.

SCHEME I

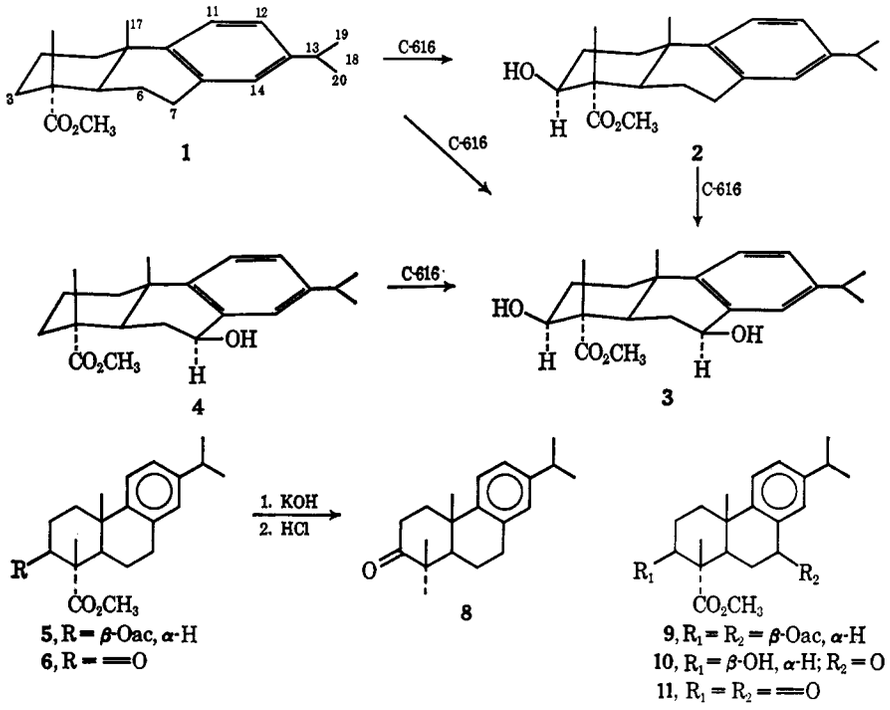


TABLE I

CHEMICAL SHIFTS[a]

Compd	C-17 Me	C-15 Me	C-3α	C-5α	C-6α	C-7α	C-11	C-12	C-14
1	1.20	1.26					7.13	7.00	6.88
1[d]	1.16	1.31							
2	1.20	1.26	m, 4.03						
2[d]	1.21	1.55							
3	1.26	1.26	m, 3.98			t, 4.80, J = 8	7.11	7.11	7.38
5	1.23	1.30	m, 5.21						
6	1.44	1.32							
8	1.26	d, 1.26, J = 7							
9	1.31	1.31	m, 5.21			t, 6.05, J = 8	(7.02, 7.16)		
10	1.26	1.31	m, 4.08						
11	1.53	1.46							
12	1.26	1.35							
13	1.30	1.38							
15	1.53	1.68	m, 4.00	d, 2.45, J = 4	d, 4.15				
16	1.55	1.55	m, 5.16	d, 2.88, J = 5	d, 5.65				
17[b]	1.65	1.65	m, 4.00	d, 2.35, J = 4	d, 4.08				
18	1.56	1.56	m, 5.16	d, 2.87, J = 5	d, 5.68				
Methyl norisopropyl-3-oxo-dehydro abietate	1.41[c]	1.30							
4	(1.26, 1.28)					t, 4.83, J = 8	7.11	7.11	7.40
Methyl 7β-acetoxydehydro-abietate	1.28	1.28				t, 6.08, J = 8	(7.01, 7.01)		

[a] Obtained in $CDCl_3$. Expressed as parts per million from TMS. J values are in cycles per second. [b] DMSO added for solubility. [c] See ref 7. [d] Obtained in pyridine-d_5.

group in the nmr spectrum of **3** and of diacetate **9** is identical in position and appearance with the C-3α proton in the nmr spectrum of **2** and **5**, respectively. Incubation of **2** under the same conditions as incubation of methyl dehydroabietate affords diol **3**, which confirms the identity of the C-3β hydroxyl group in **3** with that of **2**. No methyl 7β-hydroxydehydroabietate is found in the crude chloroform extract of the methyl dehydroabietate conversion. However, incubation of methyl 7β-hydroxydehydroabietate with *C. sasakii* does afford diol **3**, which verifies the assignment of the C-7β hydroxyl in **3**.

Selective chromate oxidation of **3** gives hydroxy ketone **10** and dione **11**. Further chromate oxidation of **10** affords **11**. Mass and nmr spectroscopy show that compound **19**, the deuterium exchange product of hydroxy ketone **10**, contains two deuteriums, and compound **20**, the deuterium exchange product of dione **11**, contains four deuteriums. The downfield shift of the C-17 methyl group in the nmr spectrum of **11** relative to **10** is similar to the 3-keto-induced shift of the C-17 methyl of ketone **6** relative to compound **2**.

Considerable starting material is recovered from incubation of methyl dehydroabietate. However, in-

SCHEME II

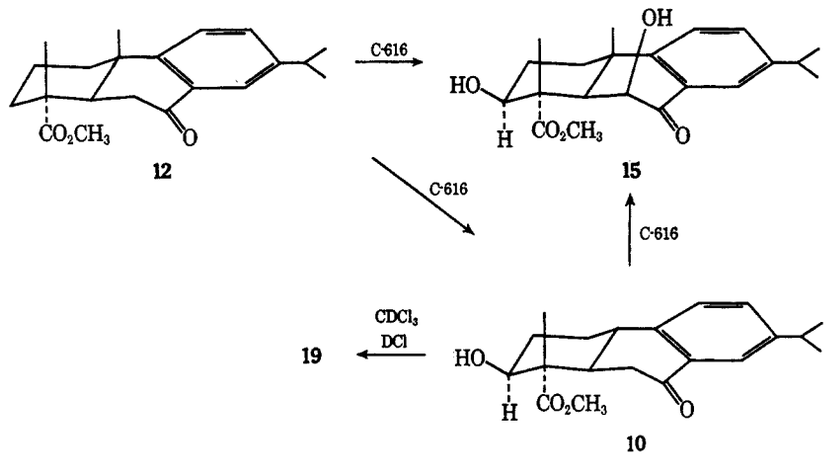
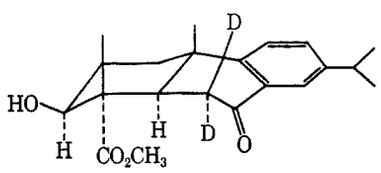


TABLE II



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Proton	Chemical shift, δ, ppm	Coupling constants, cps
3α	4.08	$J_{3\alpha-2\alpha} = 5$
2α	1.96	$J_{3\alpha-2\beta} = 10$
2β	1.78	$J_{2\alpha-2\beta} = -10$
1α	1.92	$J_{2\alpha-1\alpha} = 5$
1β	2.40	$J_{2\alpha-1\beta} = 3$
		$J_{2\beta-1\alpha} = 10$
		$J_{2\beta-1\beta} = 5$
		$J_{1\alpha-1\beta} = -11$

creased incubation time did not result in higher yields of **2** or **3**, but did diminish the amount of starting material recovered. Some unidentified highly polar material is obtained from this conversion, indicating that **3** is further oxidized by *C. sasakii*.

Incubation of methyl 7-oxodehydroabietate (**12**) with *C. sasakii* for 48 hr gives metabolite **15** (Scheme II). The infrared spectrum of **15** also shows a conjugated carbonyl and two hydroxyl absorption. The bathochromic shift in the ultraviolet spectrum of **15** upon addition of base and the instability of **15** to alumina chromatography led us to postulate that one of the hydroxyls of diol **15** is in the C-6 position. This assignment was verified by the nmr spectrum of **15**. Analogous to the introduction of a 6β hydroxyl into a 5α steroid,[11,12] the C-17 methyl signal in the nmr spectrum of **15** is shifted downfield 0.27 ppm relative to the same methyl signal for monohydroxy ketone **10**. The nmr spectra of **15** and the corresponding diacetate **14** show both the expected doublet for the 5α proton coupled to the 6α proton and a low-field proton which is identical with the 3α proton in the nmr spectra of **2** and **5**, respectively. Incubation of **10** with *C. sasaki* gives **15**. Thus the sequence of enzymatic hydroxylation of **12** to **15** is analogous to the dihydroxylation of methyl dehydroabietate to diol **3**, *i.e.*, hydroxylation in the C-3β position followed by hydroxylation in ring B. This conversion also verifies the assignment of one of the hydroxyl groups of **15** to the C-3β position. A thin layer chromatogram developed three times shows a small amount of **10** present in the crude chloroform broth extract from incubation of **12**.

Incubation of methyl 7,18-dioxodehydroabietate (**13**) with *C. sasakii* for 48 hr gives metabolite **17**. The argument for assignment of the structure depicted in Scheme III for **17** is analogous to the proof of structure for **15**. After allowing for the different C-13 substituent and its effect on the C-17 methyl group, the nmr spectra of **17** and the corresponding diacetate **18** are similar to those of **15** and **16**, respectively.

Incubation of methyl 7-oxo-18-acetoxydehydroabietate (**14**) with *C. sasakii* results in destruction of the organism within 24 hr, whereupon starting **14** is quantitatively recovered from the fermentation broth.

Examples[13] of enzymatic hydroxylation of higher terpenoids at C-3 are rare. The origin of the C-3 oxygen in terpenoids has been explained by the requirement of a squalene cyclohydroxylase for the cyclization of squalene. However, Barton and Moss[14] have proposed that biosynthetic cyclization of terpene compounds might be initiated by H^+, the oxygen function at C-3 being introduced at a later stage by hydroxylation. This hypothesis would explain the isolation of numerous di- and triterpenoids which lack an oxygen at C-3. The hydroxylation of dehydroabietanes at C-3 by fungal oxidases is consistent with the hypothesis of cyclization followed by hydroxylation. Three *Juniperus* tree dehydroabietanes cogeneric with ferruginol—hinokiol, sugiol, and proxanthoperol—contain oxygen functions in the C-3, C-6, or C-7 positions. The fungal hydroxylation of dehydroabietanes in the same positions which bear oxygen in dehydroabietanes obtained from *Juniperus* trees provides an example of a fungus and a higher plant possessing the same oxidase selectivity.

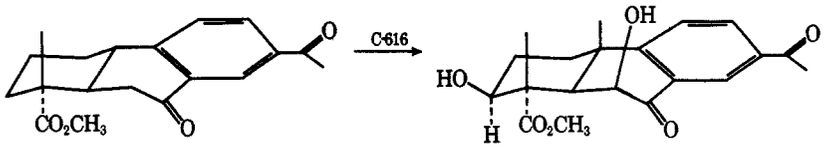
(11) D. R. Brannon, *et al.*, *J. Org. Chem.*, **32**, 1521 (1967).

(12) The similarity of the ultraviolet absorption maximum of **15** and **17** (254 mμ) to the calculated (A. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," The Macmillian Co., New York, N. Y., 1964, p 109) value of an *o,m*-dialkyl-substituted acetophenone (252 mμ) implies the near coplanarity of the C-7 carbonyl with the aromatic C ring. According to Drieding models, such a conformation of the B ring makes the C-6β position of **17** analogous to a 5α steroid with respect to the A/B angular methyl.

(13) P. C. Cherry, E. R. H. Jones, G. D. Meakins, *Chem. Commun.*, 587 (1966).

(14) D. H. R. Barton and G. P. Moss, *ibid.*, 261 (1966).

SCHEME III



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Experimental Section

Melting points were corrected. Analysis were performed by Mr. George Maciak and associates of these laboratories. Infrared spectra were recorded with a Beckman IR-7 spectrometer, ultraviolet spectra were determined with a Cary 14 recording spectrometer, nmr spectra were obtained with a Varian A-60 spectrometer with TMS (δ 0.00 ppm) as internal standard, and mass spectra were taken on a CEC 21-110A using 70 eV with a direct source inlet system. Thin layer chromatograms were run on Merck silica gel GF precoated plates, with detection by iodine vapor. Grace 950 silica gel was used for column chromatography.

General Methods of Incubation.—Flasks (500 ml) containing 200 ml of sterile solution consisting of 30 g of Difco malt extract, 20 g of reagent dextrose, 1 g of Bacto peptone, and 1000 ml of distilled water were inoculated with *C. sasakii* Lilly C-616 and incubated at 25° for 96 hr on a 250-rpm 2-in. rotary shaker. A solution of 100 mg of terpene dissolved in 0.8 ml of dimethylformamide was then added and incubation was continued for the number of hours indicated below. The flask contents were combined, and the mycelium was filtered off. The aqueous filtrate was extracted by stirring for 3 hr with a high-speed mechanical stirrer and with one-half of its volume of chloroform. The mycelium was extracted by the same procedure, and the extracts were combined unless otherwise indicated below.

Incubation of Methyl Dehydroabietate (1).—Methyl dehydroabietate (8 g) was incubated for 96 hr as described above. Removal of the chloroform from the crude broth extract gave 4.6 g of material. Extraction of the mycelium gave 1.2 g of chloroform soluble material. A thin layer chromatogram (ethyl acetate–benzene, 1:1) of the broth extract showed methyl dehydroabietate and **3** as major components. Compound **2** and some very polar material appeared as minor components. A thin layer chromatogram of the mycelial extract showed methyl dehydroabietate and **2** as major components compared with diol **3**. No spot corresponding to methyl 7β-hydroxydehydroabietate was detected in either chromatogram. Chromatography of the broth extract on 500 g of silica gel gave 2.4 g of methyl dehydroabietate upon ethyl acetate–benzene (1:1) elution.

Further elution with ethyl acetate–benzene (1:1) gave 220 mg of **2** which crystallized upon addition of hexane: mp 120–122°; ir (KBr) 3230, 1730, 830 cm^{-1}. The mass spectrum of **2** shows a parent ion at *m/e* 330 and a large P − H_2O peak at *m/e* 312.

Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.29; H, 9.25.

Further elution with ethyl acetate–benzene (1:1) gave 1.20 g of diol **3**. Recrystallization from ethyl acetate–hexane (1:3) gave mp 169–171°; ir (KBr) 3430, 1700 cm^{-1}. The mass spectrum of **3** showed a parent ion at *m/e* 346 and a large P − H_2O peak at *m/e* 328.

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.95; H, 9.00.

Elution with ethyl acetate gave 210 mg of noncrystalline material whose composition is still under investigation. Chromatography of the mycelial extract under the same conditions gave 310 mg of methyl dehydroabietate, 440 mg of **2**, and 215 mg of diol **3**, whose melting point and infrared and nmr spectra are identical with those described above.

Acetylation of Alcohol 2.—To 120 mg of **2** in 3 ml of acetic anhydride was added 3 drops of pyridine. After stirring at room temperature for 1 hr, the excess acetic anhydride was removed under vacuum, and the resulting residue was partitioned between ether and water. The ether portion, after washing with water and drying over $MgSO_4$, was concentrated to give 90 mg of crystalline **5**: mp 158–160°; ir (KBr) 1730, 1750 cm^{-1}. The mass spectrum of **5** gives a parent ion peak at *m/e* 372.

Anal. Calcd for $C_{23}H_{32}O_4$: C, 74.15; H, 8.66; O, 17.19. Found: C, 74.08; H, 8.39; O, 17.34.

Oxidation of Alcohol 2.—To 100 mg of **2** in 10 ml of acetone was added dropwise 200 mg of CrO_3 in 5 ml of acetone. After 6 hr, a thin layer chromatogram (ethyl acetate–benzene, 1:3) showed no starting material remaining. The acetone was removed under vacuum, and the resulting residue was partitioned between ether and water. The ether portion was washed with water, dried over $MgSO_4$, and then concentrated to give 71 mg of ketone **6** which crystallized upon addition of hexane: mp 99–100°; ir (KBr) 1745 cm^{-1}. The mass spectrum of **6** shows the parent ion peak at *m/e* 328.

Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.82; H, 8.60.

To 27 mg of ketone **6** in $CDCl_3$ was added 100 μl of 38% DCl in D_2O in an nmr tube. After shaking at room temperature for 2 hr, the nmr spectrum showed greater than 90% exchange of two protons. Concentration of the chloroform portion after briefly washing with water gave a crystalline material, **7**, whose mass spectrum showed the parent ion at *m/e* 330. Greater than 90% of dideuterio exchange of ketone **6** occurred as calculated from the mass spectra of **6** and **7**.

Saponification and Decarboxylation of Ketone 6.—To 25 mg of **6** in 2 ml of ethylene glycol was added 22 mg of KOH in 1 ml of water. After refluxing for 6 hr, the mixture was poured into 10 ml of water and repeatedly extracted with ether. The remaining aqueous solution was concentrated to 3 ml, 1 ml of ethanol was added, and the solution was acidified with concentrated HCl solution. After stirring for 3 hr, the solution was extracted with ether. Concentration of the ether gave 15 mg of **8** which could not be crystallized, ir (film) 1700 cm^{-1}. The mass spectrum of **8** gave a parent peak at *m/e* 284.

Reduction of Ketone 6.—To 100 mg of ketone **6** in 10 ml of ether was added 75 mg of sodium trimethoxyborohydride. After stirring at room temperature for 2 hr, the reaction mixture was filtered and the filtrate washed repeatedly with water. The ether portion was dried over $MgSO_4$ and concentrated to give 75 mg of material. A thin layer chromatogram (ethyl acetate–benzene 1:1) showed alcohol **2** to be the only component. Addition of ethyl acetate–hexane (1:1) to the material gave crystalline **2**, which is identical in all respects with that obtained from incubation of methyl dehydroabietate as described above.

Acetylation of Diol 3.—Diol **3** (50 mg) was acetylated by the same conditions as described above for the acetylation of **2**. Addition of hexane–ether (1:1) to the reaction product gave 22 mg of crystalline **9**: mp 128–130°; ir (KBr) 1730, 1750 cm^{-1}. The mass spectrum of **9** gave a parent ion peak at *m/e* 430.

Anal. Calcd for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96; O, 22.30. Found: C, 69.70; H, 7.89; O, 22.43.

Oxidation of Diol 3.—To 200 mg of **3** in 10 ml of acetone was added dropwise 75 mg of CrO_3 dissolved in 4 ml of acetone. After stirring at room temperature for 3 hr, a thin layer chromatogram (ethyl acetate–benzene 1:1) of the reaction mixture showed starting **3** and dione **11** as minor components compared with a predominent amount of hydroxy ketone **10**. The acetone was removed under vacuum and the reaction residue partitioned between ether and water. The ether portion, after washing with water and drying over $MgSO_4$, gave 160 mg of material which was chromatographed on 50 g of silica gel. Elution with ethyl acetate–benzene (1:10) gave 30 mg of dione **11**: mp 140–142°; ir (KBr) 1680, 1720, 1745 cm^{-1}. The mass spectrum of **11** gave a parent ion peak of *m/e* 342.

Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.68; H, 7.58.

Elution with ethyl acetate–benzene (1:3) gave 140 mg of **10** which could not be crystallized, but formed a gel upon addition of hexane. Vacuum filtration of the gel gave a low-melting solid: mp 70–72°; ir (KBr) 3230, 1680 cm^{-1}. The mass spectrum of **10** gave a parent ion peak at *m/e* 344.

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.91; H, 7.99.

To 35 mg of hydroxy ketone **10** in $CDCl_3$ was added 100 μl of 38% DCl in D_2O in an nmr tube. After shaking for 3 hr,

the spectrum showed approximately 90% deuterio exchange of the C-6 protons. Concentration of the chloroform portion, after briefly washing with water, gave a crystalline material, **19**, whose mass spectrum showed the parent ion peak at *m/e* 346. Approximately 95% of dideuterio exchange of hydroxy ketone **10** occurred as calculated from the mass spectra of **10** and **19**.

Oxidation of Hydroxy Ketone 10.—To 50 mg of **10** in 3 ml of acetone was added dropwise 25 mg of CrO_3 in 2 ml of acetone. A thin layer chromatogram (ethyl acetate–benzene 1:1) of the reaction mixture showed complete conversion of **10** into dione **11** after stirring at room temperature for 3 hr. The usual work-up afforded 31 mg of dione **11** whose spectral and physical properties are identical with those of **11** described above. To 20 mg of dione **11** in $CDCl_3$ was added 200 μl of 38% DCl in D_2O in an nmr tube. After shaking for 2 hr, the nmr spectrum showed the loss of four protons. Concentration of the chloroform portion gave crystalline **20** whose mass spectrum showed the parent ion peak at *m/e* 346. Approximately 95% tetradeuterio exchange occurred as calculated from the mass spectrum.

Incubation of Alcohol 2.—A total of 169 mg of **2** was incubated for 48 hr as described above. Chromatography of the combined broth and mycelial chloroform extracts on 20 g of silica gel gave 21 mg of starting alcohol **2** upon elution with ethyl acetate–benzene (1:1).

Further elution gave 117 mg of crystalline **3**, mp 165–167°, whose spectral properties are identical with those described above for **3**.

Incubation of Methyl 7β-Hydroxydehydroabietate (4).—A total of 250 mg of methyl 7β-hydroxydehydroabietate was incubated for 4 days as described above. Chromatography of the combined broth and mycelial chloroform extracts on 50 g of silica gave 80 mg of starting material upon elution with ethyl acetate–benzene (1:1). Further elution with the same solvent gave 90 mg of crystalline **3**, mp 167–168°. Elution with ethyl acetate gave 30 mg of unidentified polar material.

Incubation of Ketone 12.—Ketone **12** (5 g) was incubated for 2 days as described above. Chloroform extraction of the broth gave 2.6 g of material whose thin layer chromatogram (ethyl acetate–benzene, 1:1) showed starting ketone **12**, diol **15**, and several minor components more polar than **15**. A thin layer chromatogram of the mycelial chloroform extract (980 mg) showed the same components, except ketone **12** was the most predominant component. Chromatography of the combined extracts on 250 g of silica gel gave 800 mg of starting ketone **12** upon elution with ethyl acetate–benzene (1:1).

Further elution with the same solvent gave 2.3 g of crude **15**. Two recrystallizations from ethyl acetate gave an analytical sample: mp 187–188°; ir (KBr) 3340, 3480, 1710, 1605 cm^{-1}; uv max (EtOH) 254 mμ (ε 11,400), which shifted to 375 mμ upon addition of base. The ultraviolet spectrum of **12** gave uv max (EtOH) 254 mμ (ε 10,600), which did not shift upon addition of base. The mass spectrum of **15** gave a parent ion peak at *m/e* 360.

Anal. Calcd for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83; O, 22.20. Found: C, 69.86; H, 8.00; O, 22.22.

A thin layer chromatogram developed three times (ethyl acetate–benzene, 1:9) of the crude transformation mixture run along with **10** showed **10** present in the mixture as a minor component.

Incubation of Hydroxy Ketone 10.—Hydroxy ketone **10** (90 mg) was incubated for 48 hr as described above. A thin layer chromatogram (ethyl acetate–benzene, 1:9) developed three times showed that the mycelial extract contained **15**. The broth extract contained considerable starting material. Chromatography of the mycelial extract on 25 g of silica gel gave 35 mg of crystalline **15**, mp 182–183°, whose infrared spectrum was identical with that of **15** obtained above from incubation of **12**.

Acetylation of 15.—To 50 mg of metabolite **15** in 3 ml of acetic anhydride was added 3 drops of pyridine. After stirring for 12 hr at room temperature, the acetic anhydride was removed under vacuum and the residue was partitioned between water and ether. The ether portion gave 52 mg of material which was chromatographed on 25 g of silica gel. Elution with ethyl acetate–benzene (1:9) gave **16**. All attempts to crystallize **16** gave only amorphous material.

Incubation of Diketone 13.—Diketone **13** (5 g) was incubated for 2 days as described above. Analogous to the incubation of **12** described above, tlc showed that the mycelial extract (1.10 g) contained largely starting material, whereas the broth extract (3.2 g) contained **17** as the major component. Chromatography of the combined extracts on 250 g of silica gel gave 1.22 g of starting diketone **13** upon elution with ethyl acetate–benzene (1:1).

Further elution with the same solvent gave 2.1 g of crude metabolite **17**. Crystallization from ethyl acetate gave pure **17**: mp 193–195°, ir (KBr) 3340, 3480, 1717, 1605 cm^{-1}; uv max (EtOH) 254 mμ (ε 15,000), which shifted to 375 mμ upon addition of base. The mass spectrum of **17** gave the parent ion peak at *m/e* 360.

Anal. Calcd for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71; O, 26.64. Found: C, 66.57; H, 6.98; O, 26.64.

Acetylation of 17.—Diol **17** (50 mg) was acetylated under the same conditions as described above for the acetylation of **17**. Addition of ether to the crude reaction product gave crystalline **18**, mp 199–200°.

Anal. Calcd for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35; O, 28.80. Found: C, 65.11; H, 6.41; O, 28.50.

Registry No.—**1**, 1235-74-1; **2**, 17751-30-3; **3**, 17751-32-5; **4**, 17751-34-7; **5**, 17831-48-0; **6**, 17831-49-1; **8**, 16898-96-7; **9**, 17831-51-5; **10**, 17751-38-1; **11**, 17831-53-7; **12**, 17751-36-9; **13**, 5335-63-7; **15**, 17810-49-0; **16**, 17831-57-1; **17**, 17831-58-2; **18**, 17831-59-3; methyl norisopropyl-3-oxodehydroabietate, 1686-52-8; methyl 7β acetoxydehydroabietate, 17901-36-9.

Acknowledgments.—We are indebted to Dr. Thomas F. Sanderson, Hercules Inc., Wilmington, Del., for numerous diterpene samples and to the Physical Chemistry Department of these laboratories for spectral measurements. We also thank Professor Ernest Wenkert, Indiana University, Bloomington, Ind., and Dr. Marvin Gorman of these laboratories for helpful discussions.