Metabolites of (+)-Dehydroabietic Acid in Rabbits

TAKASHI MATSUMOTO*, NORIAKI HAYASHI*, TAKASHI ISHIDA§, AND YOSHINORI ASAKAWA‡x

Received October 18, 1988, from the *Department of Chemistry, Faculty of Sciences, Hiroshima University, Higashisenda-machi, Nakaku, Hiroshima 770, the §Hiroshima Institute of Technology, 2-1-1 Miyake, Saekiku, Hiroshima 731-51, and the ‡Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan. Accepted for publication September 11, 1989.

Abstract □ The seven metabolites of (+)-dehydroabietic acid (DHA) were newly isolated from rabbit urine by liquid chromatography. On the basis of chemical and spectral data their structures were established to be (15*S*)-8,11,13-abietatrien-16,18-dioic acid, 2α-hydroxy-8,11,13,15-abietatetraen-18-oic acid, (15*R*)-15,16-dihydroxy-8,11,13-abietatrien-18-oic acid, 2β,15-dihydroxy-8,11,13-abietatrien-18-oic acid, (15*S*)-2β,16-dihydroxy-8,11,13-abietatrien-18-oic acid, 2α,15-dihydroxy-8,11,13-abietatrien-18-oic acid, and (15*S*)-2α,16-dihydroxy-8,11,13-abietatrien-18-oic acid. The possible hydroxylation routes of DHA in rabbits and the difference between the metabolism of DHA in micro-organisms and that in rabbits are discussed.

We have systematically studied the biotransformation of mono- and sesquiterpenoids in rabbits and reported their hydroxylation routes and isolation of insect pheromones, mushroom component, and flavorous compounds from their metabolites.[1–8] Diterpene resin acids, arising from softwoods used in the pulping industry, have been shown to be the major toxicants in kraft pulp mill effluent.[9–11] Among the toxic resin acids identified are dehydroabietic acid (DHA) and abietic acid. Recently, Mattsoff et al.[12] reported that DHA causes red cell breakdown to bring out jaundice in rainbow trout. Kutney et al.,[13] Kieslich,[14] and Ekman et al.[15] reported the biotransformation of DHA in various micro-organisms. On the other hand, the glycerin ester of DHA is now widely used as the base of chewing gum with vinyl acetate resin, and is taken into our bodies.[16] In order to assess the potential toxicity of such food additives, it is important to establish the biotransformation of DHA and its related compounds. However, little attention has been paid to biotransformation of such diterpenoids in mammals. Previously, we isolated three metabolites (as methyl esters), methyl 8,11,13,15-abietatetraen-18-oate (**1**), methyl 15-hydroxy-8,11,13-abietatrien-18-oate (**2**), and methyl 16-hydroxy-8,11,13-abietatrien-18-oate (**8**) after administration of (+)-sodium dehydroabietate.[6] Recently, we carried out the synthesis of methyl (15*S*)-16-hydroxy-8,11,13-abietatrien-18-oate (**18a**) and its (15*R*) epimer (**18b**) and confirmed that the absolute configuration at C-15 in metabolite **8** possesses an *S*-configuration.[17]

As an extension of the previous work, we further studied the metabolites of DHA and isolated seven additional compounds and characterized their structures by chemical and spectral evidence. We report here the structures of the new metabolites, and discuss the possible hydroxylation routes of DHA in rabbits and the difference between the metabolism of DHA in micro-organisms and that in rabbits.

Results and Discussion

Commercial sodium dehydroabietic acid (DHA) was administered orally to rabbits and the metabolites (after the urine was enzymatically degraded by β-glucuronidase:arylsulfatase and extracted with ether) were methylated with diazomethane to give a pale brown oil (40% recovery). The absence of any metabolites possessing the DHA skeleton in the ethyl ether non-extract aqueous solution was confirmed by the ^{1}H NMR spectrum of the ether extract after acidification of the aqueous layer. The methylated product was chromatographed on silica gel to give seven compounds, **A** (=**7**), **B** (=**6**), **C** (=**5**), **D** (=**3**), **E** (=**9**), **F** (=**4**), and **G** (=**10**), in addition to the previously known compounds methyl 8,11,13,15-abietatetraen-18-oate (**1**), methyl 15-hydroxy-8,11,13-abietatrien-18-oate (**2**), and methyl 16-hydroxy-8,11,13-abietatrien-18-oate (**8**).[6] The yield of each isolated metabolite for the administered DHA is shown in Table I. The recovery of the metabolites of DHA is estimated to be 14%. This value is considered to be extremely small, but the same phenomenon has been found in the metabolites of limonene[21] and β-ionone[23] in rabbits, as shown in Table II.

Compound A (= 7)—A combination of elemental analysis and the mass spectrum (M^+ at *m/z* 358) established the molecular formula $C_{22}H_{30}O_4$. The ^{1}H NMR spectrum (Tables III–VI) suggested that one of isopropyl methyl groups of methyl dehydroabietate (**11**) was replaced by a methoxycarbonyl group. Reduction of **7** with $LiAlH_4$ gave a diol (**12a**); this was followed by benzoylation with 4-nitrobenzoyl chloride to afford a bis(4-nitrobenzoate) whose physical and spectral data were identical with those of (15*S*)-16,18-bis(4-nitrobenzoyloxy)-8,11,13-abietatrien (**13a**) prepared from authentic methyl (15*S*)-16-hydroxy-8,11,13-abietatrien-18-oate (**8a**).[17] For direct comparison with the (15*S*)-isomer (**13a**), (15*R*)-16,18-bis(4-nitrobenzoyloxy)-8,11,13-abietatrien (**13b**)[17] was also prepared from authentic **8b** via (15*R*)-16,18-diol (**12b**). The synthetic (15*R*)-isomer (**13b**) was not identical with the natural derivative (**13a**). Thus, the structure of compound **A** was established to be dimethyl (15*S*)-8,11,13-abietatrien-16,18-dioate (**7**) and the corresponding (15*S*)-8,11,13-abietatrien-16,18-dioic acid (**7′**) was obtained as a metabolite of DHA.

Compound B (=6)—The molecular formula $C_{21}H_{28}O_3$ was established by a combination of the mass spectrum (M^+, at *m/z* 328) and elemental analysis. The ^{1}H NMR spectrum (Tables III–VI) suggested the presence of a secondary hydroxyl group and an isopropyl group. Catalytic hydrogenation of **6** afforded a dihydro derivative (**14**) which was acetylated

Table I—Yields of Isolated Metabolites, Methyl Esters, and Solvents

Isolated Compound	Yield, mg (Recovery, %)	Solvent
1	273 (2.28)	Hexane:benzene (1:1)
A(=7)	290 (2.42)	Benzene
2	179 (1.49)	Benzene:ether (9:1)
8a	307 (2.56)	Benzene:ether (9:1)
B(=6)	74 (0.62)	Benzene:ether (9:1)
C(=5)	388 (3.23)	Benzene:ether (4:1)
D(=3)	54 (0.45)	Benzene:ether (7:3)
E(=9)	24 (0.20)	Benzene:ether (7:3)
F(=4)	65 (0.54)	Benzene:ether (3:2)
G(=10)	32 (0.27)	Benzene:ether (1:1)

0022-3549/90/0600-0540$01.00/0

Table II—Recovery of Metabolites of Terpenoids and Flavone in a Few Mammals

Sample	Animal	Dose, g	Metabolite, g	Recovery, %	Reference
DHA	Rabbit	12	1.686	14.0	
Cedrol	Rabbit	1	0.52	52	18
p-Cymene	Rat	0.075	—[a]	80	19
	Guinea pig	0.090–0.105	—[a]	71	19
Flavone	Rat	0.092–0.108	—[a]	28[b]	20
Limonene	Rabbit	25	4.35	17.4	21
β-Ionone	Rabbit	23	1.72	7.5	22

[a] No metabolite recovered data are given. [b] Excretion of radioactivity in the urine.

Table III—Proton Nuclear Magnetic Resonance Spectral Data of Compounds A–G

Proton	A (=7)	B (=6)	C (=5)	D (=3)	E (=9)	F (=4)	G (=10)
H-2α	—	—	—	4.37 qi (J = 4)	4.38 qi (J = 4)	—	—
H-2β	—	3.85–4.25	—	—	—	3.90–4.20	3.80–4.30
H-7	—	—	—	2.90 dd (J = 8,4)	2.75–3.00 (2H)	2.91 dd (2H, J = 8.4)	2.75–3.05 (2H)
H-11	7.18 d (J = 8)	7.12 bs	7.08 bs	7.12 bs	7.20 d (J = 8)	7.12 bs	7.20 d (J =8)
H-12	7.00 dd (J = 8.2)	7.21 bs (2H)[c]	7.18 (2H)[c]	7.20 (2H)[c]	6.98 dd (J = 8.2)	7.20 (2H)[c]	6.98 dd (J = 8.2)
H-14	6.92 bs	—[c]	—[c]	—[c]	6.85 bs	—[c]	6.88 bs
H-15	3.62 q (J = 7)	—	—	—	2.75–3.00 (1H)	—	2.75–3.05
H-16	3.65 (C_{15}–CO_2Me)	5.00 q (1H, J = 1.5) 5.30 bs (1H)	3.65 s (2H)	1.52 s (3H)	3.67 (2H)	1.52 s (3H)	3.67 *d* (2H, J = 7)
H-17(C_{15}-Me)	1.43 d (J = 7)	2.10 s	1.47 s	1.52 s	1.21 d (J = 7)	1.52 s	1.21 d (J = 7)
H-18(C_4-CO_2Me)	3.65 s	3.68 s	3.65 s	3.68 s	3.67 s	3.66 s	3.67 s
H-19(C_4-Me)	1.25 s	1.28 s[a]	1.25 s	1.45 s	1.45 s	1.28 s[a]	1.28[a]
H-20(C_{10}-Me)	1.18 s	1.22 s[a]	1.18 s	1.45 s	1.45 s	1.21 s[a]	1.21[a]

[a,b] Assignments may be changeable, respectively (Tables III–VI). [c] This signal is superposed into another aromatic proton signal.

Table IV—Proton Nuclear Magnetic Resonance Spectral Data of Compounds 12–15

Proton	12a	12b	13a	13b	14	15
H-2α	—	—	—	—	—	2.05 s (3H, OAc)
H-2β	—	—	—	—	4.00—4.30	5.00–5.45
H-7	2.70–3.00	2.70–3.00	—	—	2.75–3.00	—
H-11	7.18 d (J = 8)	7.18 d (J = 8)	7.22 d (J = 8)	7.23 d (J = 8)	7.17 d (J = 9)	7.13 d (J = 8)
H-12	6.92 bd (J = 8)	6.93 dd (J = 8.2)	7.02 bd (J = 8)	7.03 bd (J = 8)	6.97 bd (J = 9)	6.98 dd (J = 8.2)
H-14	6.85 bs	6.85 bs	6.94 bs	6.92 s	6.88 bs	6.87 bs
H-15	2.70–3.00	2.70–3.00	3.19 m	3.18 m	2.75—3.00	—
H-16	3.62 d (2H, J = 7) 1.67 s (OH)	3.62 d (2H, J = 7)	4.40 dd (2H, J = 7.2)	4.40 dd (2H, J = 7.2)	1.21 d (3H, J = 7)	1.20 d (3H, J = 7)
H-17 (C_{15}–Me)	1.21 d (J = 7)	1.21 d (J = 7)	1.37 d (J = 7)	1.38 d (J = 7)	1.21 d (J = 7)	1.20 d (J = 7)
H-18(C_4–CO_2Me)	3.16 d (1H, J = 11)[c] 3.44 d (1H, J = 11)[c] 1.67 s (OH)	3.16 d (1H, J = 11)[c] 3.44 d (1H, J = 11)[c]	4.04 d (1H, J = 11)[c] 4.29 d (1H, J = 11)[c] 8.02–8.30[d]	4.04 d (J = 11)[c] 4.29 d (J = 11)[c] 8.05–8.35[d]	3.67 s	3.67 s
H-19(C_4-Me)	0.85 s	0.85 s	1.05 s	1.06 s	1.28 s[a]	1.33 s[a]
H-20(C_{10}-Me)	1.18 s	1.18 s	1.23 s	1.24 s	1.21 s[a]	1.27 s[a]

[a,b] Assignments may be changeable, respectively (Tables III–VI). [c] C_4—CH_2O—. [d] $C_6H_4NO_2$.

to give an acetate (15). Compound 14 was identical with methyl 2α-hydroxy-8,11,13-abietatrien-18-oate,[13] which is described later. Thus, the structure of compound B was established to be methyl 2α-hydroxy-8,11,13,15-abietatetraen-18-oate (6) and the corresponding 2α-hydroxy-8,11,13,15-abietatetraen-18-oic acid (6′) was obtained as a metabolite of DHA.

Compound C (=5)—The mass spectrum (M^+, m/z 346) established the molecular formula $C_{21}H_{30}O_4$. The ^1H NMR spectrum (Tables III–VI) suggested an introduction of two hydroxyl groups (primary and tertiary) on the isopropyl group. The other signals were similar to those of 2. To determine the absolute configuration of 5, the following correlation was carried out. Mesylation of 5 gave a monomesylate (17) which when treated with sodium iodide in the presence of potassium carbonate afforded an epoxide (18). This epoxide was then submitted to catalytic hydrogenolysis using Pd–C and NaOH to give the known methyl (15*R*)-16-hydroxy-8,11,13-abietatrien-18-oate (18b).[17] Mitsui et al.[23] have reported that the hydrogenolysis of α-alkylstyrene oxides proceeded with predominant inversion of the configuration using a Pd–C catalyst in the presence of a base.

Table V—Proton Nuclear Magnetic Resonance Spectral Data of Compounds 18, 19, 20, 22, 23, and 27

Proton	18	19	20	22	23	27
H-2α	—	5.21 qi (J = 4)	—	5.31 qi (J = 4)	5.31 qi (J = 4)	5.26 m
H-2β	—	2.00 s (3H, OAc)	—	2.04 s (3H, OAc)	2.06 s (3H, OAc)[b]	3.03 s (3H, OMs)
H-7	2.70–3.10 (2H)	—	—	2.80–3.00 (2H)	2.80–3.20 (2H)	—
H-11	7.00 bs	7.00 bs	7.00–7.35 (3H)[c]	7.10 d (J = 8)	7.12 d (J = 8)	6.85–8.20 (3H)[c]
H-12	7.16 bs (2H)[c]	7.06 bs (2H)[c]	—[c]	6.95 dd (J = 8.2)	6.92 dd (J = 8.2)	—[c]
H-14	—[c]	—[c]	—[c]	6.86 bs	6.87 bs	—[c]
H-15	—	—	—	2.80–3.00	2.80–3.20	—
H-16	2.70–3.10 (2H)	1.42 s (3H)	1.53 s (3H)	1.18 d (3H, J = 7)	4.09 dd (2H, J = 7.2) 2.01 s (3H, OAc)[b]	4.34 d (2H, J = 7)
H-17(C_{15}-Me)	1.69 s	1.42 s	1.53 s (3H)	1.18 d (J = 7)	1.23 d (J = 7)	1.35 d (J = 7)
H-18(C_4-CO_2Me)	3.67 s	3.62 s	3.72 s	3.67 s	3.67 s	3.69 s
H-19(C_4-Me)	1.28 s[a]	1.36 s[a]	1.28 s[a]	1.41 s[a]	1.41 s[a]	1.46 s[a]
H-20(C_{10}-Me)	1.20 s[a]	1.33 s[a]	1.22 s[a]	1.36 s[a]	1.37 s[a]	1.43 s[a]
Benzoyl						6.85–8.20 (5H)

[a,b] Assignments may be changeable, respectively (Tables III–VI). [c] This signal is superposed into another aromatic proton signal.

Table VI—Proton Nuclear Magnetic Resonance Spectral Data of Compounds 29a, 29b, 31, 32, and 36

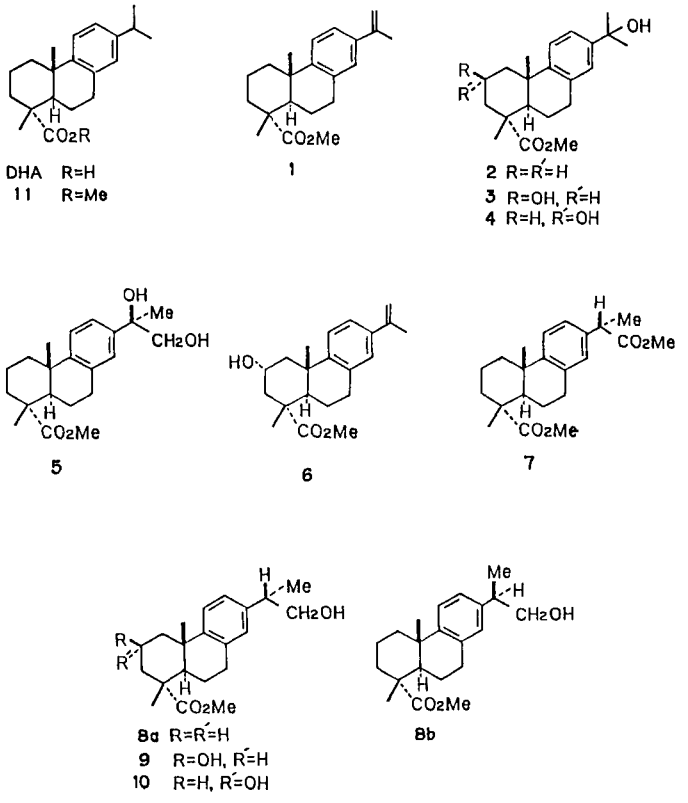
Proton	29a	29b	31	32	36
H-2α	—	—	2.06 s (3H, OAc)	2.01 s (3H, OAc)[b]	3.08 s (3H, OMs)
H-2β	—	—	5.00–5.40	5.00–5.40	—
H-7	—	—	2.92 dd (2H, J = 8.4)	2.80–3.20 (2H)	—
H-11	7.18 d (J = 8)	7.18 d (J = 8)	7.18 bs (3H)[c]	7.12 d (J = 8)	6.85–8.20 (3H)[c]
H-12	7.00 dd (J = 8.2)	7.00 dd (J = 8.2)	—[c]	6.92 bd (J = 8)	—[c]
H-14	6.86 bs	6.92 bs	—[c]	6.88 bs	—[c]
H-15	3.14 m	3.14 m	—	2.80–3.20	—
H-16	4.28 dd (1H, J = 11.7) 4.43 dd (1H, J = 11.7)	4.28 dd (1H, J = 11.7) 4.43 dd (1H, J = 11.7)	1.53 s (3H)	4.10 dd (2H, J = 7.2) 2.04 s (3H, OAc)[b]	4.35 d (2H, J = 7)
H-17(C_{15}-Me)	1.36 d (J = 7)	1.36 d (J = 7)	1.53 s	1.23 d (J = 7)	1.38 d (J = 7)
H-18(C_4-CO_2Me)	3.65 s	3.65 s	3.68 s	3.68 s	3.70 s
H-19(C_4-Me)	1.26 s	1.26 s	1.33 s[a]	1.32 s[a]	1.38 s[a]
H-20(C_{10}-Me)	1.19 s	1.19 s	1.28 s[a]	1.27 s[a]	1.32 s[a]
Benzoyl	7.25–7.60 (3H) 7.98 dd (2H, J = 8.2)	7.25–7.60 (3H) 7.98 dd (2H, J = 8.2)	—	—	6.85–8.20 (5H)

[a,b] Assignments may be changeable, respectively (Tables III–VI). [c] This signal is superposed into another aromatic proton signal.

Application of Mitsui's rule to the epoxide 18 suggested that the stereochemistry of C-15 in 18 was the R configuration. Thus, the structure of compound C was characterized to be methyl (15R)-15,16-dihydroxy-8,11,13-abietatrien-18-oate (5) and the corresponding (15R)-15,16-dihydroxy-8,11,13-abietatrien-18-oic acid (5′) was obtained as a metabolite of DHA.

Compound D (=3)—The mass spectrum (M^+ at m/z 346) established a molecular formula of $C_{21}H_{30}O_4$. The ^1H NMR spectrum (Tables III–VI) indicated the presence of secondary and tertiary hydroxyl groups. The secondary hydroxyl group of 3 was confirmed by acetylation and oxidation to give a monoacetate (19) and a carbonyl compound (20), respectively. In the ^1H NMR spectrum of 3, the appearance of the six proton singlets in very low field (δ 1.45) suggested a 1,3-diaxial-*cis*-relationship between the hydroxyl group and the methyl groups at C-4 and C-10. The splitting pattern of the methine proton signal at δ 4.37 also suggested the presence of a 2β-hydroxyl group at C-2. Catalytic hydrogenation of 3 over Pd–C in ethyl acetate in the presence of perchloric acid gave a hydrogenolyzed hydroxyester (21) which afforded an acetate (22) by acetylation with acetic anhydride in pyridine. Thus, the structure of compound D was established to be 2β,15-dihydroxy-8,11,13-abietatrien-18-oate (3) and the corresponding 2β,15-dihydroxy-8,11,13-abietatrien-18-oic acid (3′) was obtained as a metabolite of DHA.

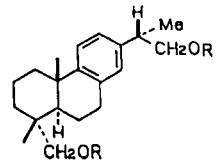
Compound E (=9)—A molecular formula of $C_{21}H_{30}O_4$ was established by the mass spectrum (M^+, at m/z 346). The ^1H NMR spectrum (Tables III–VI) indicated that two hydroxyl groups must be placed at C-2β and C-16. In order to determine



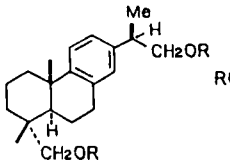
the absolute configuration at C-15, the following correlation was carried out. Compound **9** was converted into a diacetate (**23**) which was partially hydrolyzed into a 16-hydroxy compound (**24**). After a series of reactions on **24** [preparation of benzoate (**25**), partially hydrolyzed product (**26**), mesylate (**27**), demesylated product (**28**), and catalytic hydrogenation product (**29a**)], **29a** was further converted into a 4-nitrobenzoate (**30**) by alkaline hydrolysis and 4-nitrobenzoylation. The physical and spectral data of **30** were identical to those of authentic methyl (15*S*)-16-(4-nitrobenzoyloxy)-8,11,13-abietatrien-18-oate.[17] Thus, the structure of compound **E** was characterized to be methyl (15*S*)-2β,16-dihydroxy-8,11,13-abietatrien-18-oate (**9**) and the corresponding (15*S*)-2β,16-dihydroxy-8,11,13-abietatrien-18-oic acid (**9'**) was obtained as a metabolite of DHA.

Compound F (=4)—The mass spectrum (M^+ at *m/z* 346) established a molecular formula of $C_{21}H_{30}O_4$. The ^1H NMR spectrum (Tables III–VI) showed the presence of secondary and tertiary hydroxyl groups. Oxidation of **4** afforded a carbonyl compound whose spectral data were identical with those of **20**. Compound **4** afforded a monoacetate (**31**) whose ^1H NMR spectral data was slightly different from that of **19**, suggesting that the configuration of the C-2 hydroxyl group was different from that of **19**. Catalytic hydrogenolysis of **4** in the presence of perchloric acid gave methyl 2α-hydroxy-8,11,13-abietatrien-18-oate (**14**). Thus, the structure of compound **F** was established to be methyl 2α,15-dihydroxy-8,11,13-abietatrien-18-oate (**4**) and the corresponding 2α,15-dihydroxy-8,11,13-abietatrien-18-oic acid (**4'**) was obtained as a metabolite of DHA.

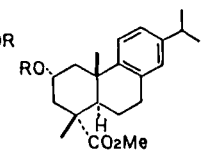
Compound G (=10)—The mass spectrum (M^+ at *m/z* 346) established a molecular formula of $C_{21}H_{30}O_4$. The presence of a primary hydroxyl group at C-16 and a secondary hydroxyl group at C-2 was suggested by the ^1H NMR spectrum. To determine the absolute configuration of C-15, the diacetate (**32**) was transformed into a benzoate (**29a**) via **33**, **34**, **35**, **36**, and **28**. The physical and spectral data of **29a** were identical with those of methyl (15*S*)-16-benzoyloxy-8,11,13-abietatrien-18-oate which was prepared from authentic methyl



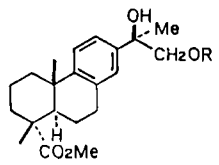
12a R=H
13a R=COC$_6$H$_4$NO$_2$(p)



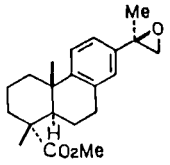
12b R=H
13b R=COC$_6$H$_4$NO$_2$(p)



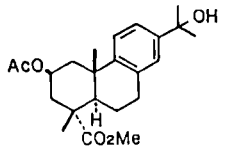
14 R=H
15 R=Ac



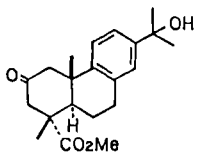
16 R=COC$_6$H$_4$NO$_2$(p)
17 R=Ms



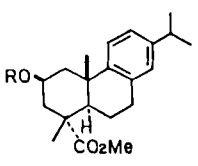
18



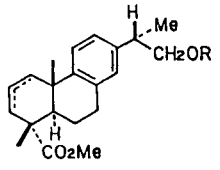
19



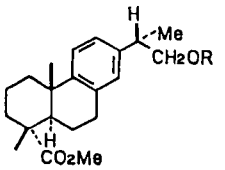
20



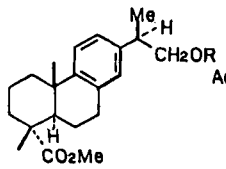
21 R=H
22 R=Ac



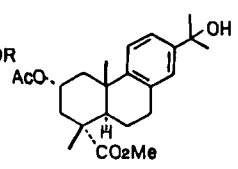
28 R=COC$_6$H$_5$



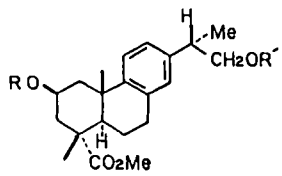
29a R = COC$_6$H$_5$
30 R = COC$_6$H$_4$NO$_2$(p)



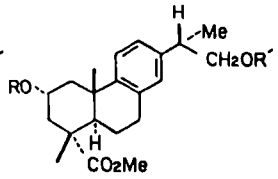
29b R = COC$_6$H$_5$



31



23 R=R'=Ac
24 R=Ac, R'=H
25 R=Ac, R'=COC$_6$H$_5$
26 R=H, R'=COC$_6$H$_5$
27 R=Ms, R'=COC$_6$H$_5$

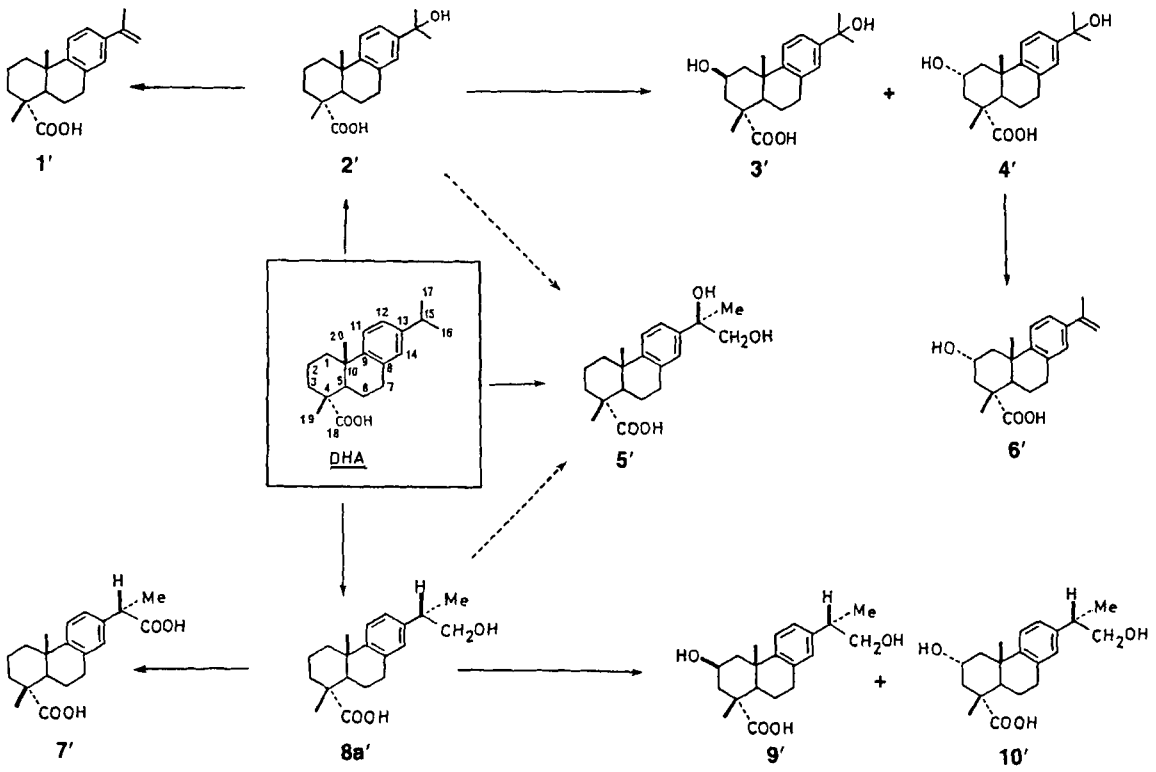


32 R=R'=Ac
33 R=Ac, R'=H
34 R=Ac, R'=COC$_6$H$_5$
35 R=H, R'=COC$_6$H$_5$
36 R=Ms, R'=COC$_6$H$_5$

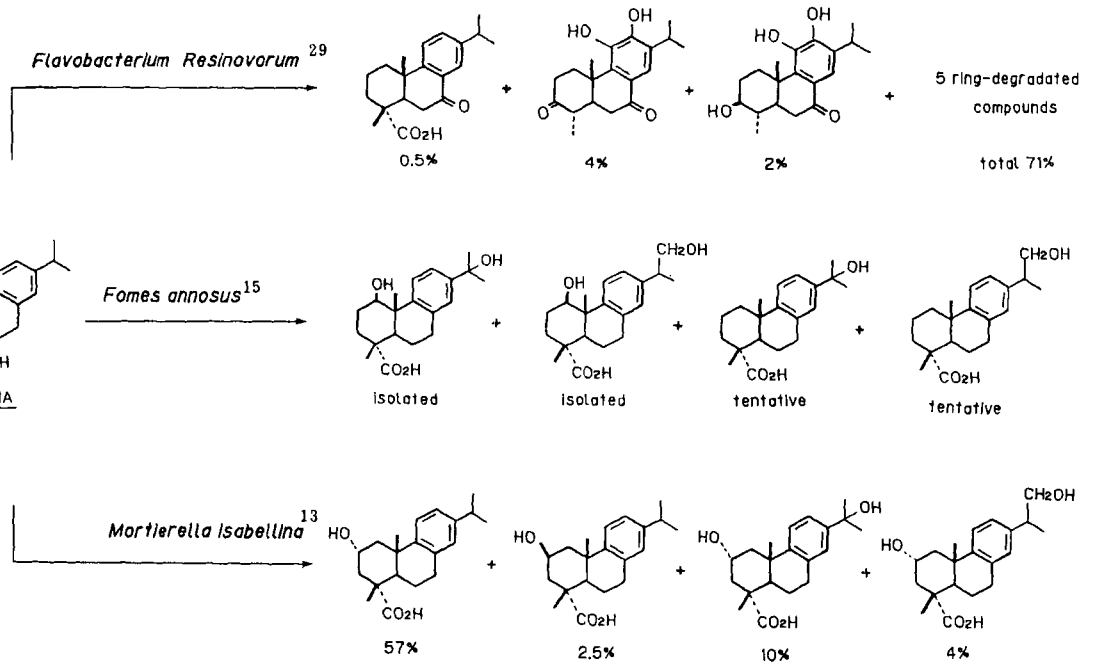
(15*S*)-16-hydroxy-8,11,13-abietatrien-18-oate (**8a**).[17] For the direct comparison with (15*S*)-benzoate (**29a**), the (15*R*)-isomer, methyl (15*R*)-16-hydroxy-8,11,13-abietatrien-18-oate (**19b**), was also prepared from authentic methyl (15*R*)-16-hydroxy-8,11,13-abietatrien-18-oate (**8b**).[17] The synthetic (15*R*)-benzoate (**19b**) was not identical with the natural derivative **29a**. Thus, the structure of compound **G** was assigned to be methyl (15*S*)-2α,16-dihydroxy-8,11,13-abietatrien-18-oate (**10**) and the corresponding (15*S*)-2α-dihydroxy-8,11,13-abietatrien-18-oic acid (**10'**) was obtained as a metabolite of DHA.

Biotrasformation of aromatic hydrocarbons has been studied extensively in relation to carcinogenic activity. In the metabolism of *p*-cymene,[3,19,24] cumene,[24–26] or isopropylbiphenyl,[27,28] having a common isopropyl group on the benzene ring, ω- and ω-1 oxidations were reported without stereochemistry. (+)-Dehydroabietic acid (DHA) also has an isopropyl group on the benzene ring and it was metabolized into 10 compounds in rabbits. The stereochemistry of seven compounds was established (Scheme I). This study establishes the metabolic end products. It is suggested that the oxidation of DHA in rabbits occurred first in C-15 and C-16 to give the metabolites **2'** and **8a'**. New data are presented which indicate that **5'** is formed directly from DHA; alternatives from **2'** and **8'** are possible. The hydroxymethyl group at C-15 in **8a'** was then oxidized to afford the carboxylic acid (**7'**). Further oxidation of **2'** and **8a'** occurred at C-2 to give the metabolites **3'**, **4'**, **9'**, and **10'**, respectively. The isolation of **7'**, **8a'**, **9'**, and **10'**, possessing the same *S*-configuration at C-15 indicated that ω-hydroxylation of the isopropyl group in DHA occurred stereoselectively in the *pro-R* methyl group. The tetraene compounds, **1'** and **6'**, are probably artifacts which might be produced by the dehydration of tertiary hydroxyl group at C-15 in **2'** and **4'** during the extraction of the crude metabolites under acidic conditions.

The metabolism of DHA in micro-organisms has been reported, and some examples are shown in Scheme II[13,15,29] In micro-organisms, each ring of DHA was oxidized to give the major metabolites. The same metabolites (**2'**, **4'**, **5'** or **7'**, **10'** or its epimer) as those isolated from rabbit urine have been found in *Mortierella isabellina*[13] and *Fomes annosus*.[15] On the other hand, in rabbits, the isopropyl group on the benzene ring of DHA was preferentially oxidized to give the major metabolites.



Scheme I



Scheme II

Experimental Section

Melting points were taken on a Yanagimoto apparatus and are uncorrected. Mass spectra were measured at an ionization potential of 70 eV. The IR spectra and optical rotation were measured in $CHCl_3$. The ^1H NMR spectra were recorded on a Hitachi R-22 spectrometer using $CDCl_3$ at 90 MHz with TMS as an internal standard, unless otherwise stated.

Application—Rabbits (Japanese White Strain, six male rabbits, each 2.5–3.0 kg) were used as experimental animals because of their easy handling and simple dosing. Commercial sodium abietate (Arakawa Chemical Industry, total 12 g) suspended in sodium sorbitate (Tween 80) solution (0.1 g/mL of 0.02% aq. sodium sorbitate) was administered orally at a dose of 2 g/rabbit to six rabbits after 1 day of starvation. After administration, food (Oriental Rabbits Foods, CR-2) and water were given to rabbits freely. For isolation of the metabolites, each rabbit was kept in a urine–feces separated metabolism cage. Urine was collected daily for 3 days under a toluene layer at room temperature, mixed together (24–72 h), centrifuged to

remove contaminants (hairs and feces), and stored at 0 °C before analysis.

Metabolite Extraction—The urine was adjusted to pH 4.0 with phosphate buffer, enzymatically degraded by β-glucuronidase:arylsulfatase (β-glucuronidase activity: ~300,000 Fishman Units/1 mL of urine; arylsulfatase activity: ~2,400,000 Roy units/1 mL of urine; Boehringer–Mannheim, West Germany; 3 mL of mixed enzyme/1 L of fresh urine) for 48 h to avoid vigorous hydrolysis conditions, and extracted at pH 2.0 in a liquid–liquid extraction apparatus (Mitamura, Tokyo) with diethyl ether for 48 h. The extract was washed successively with dilute HCl and water and dried over Na_2SO_4. Then, the metabolites were methylated with CH_2N_2 in the usual manner.

Chromatography—The mixture of methylated metabolites (5.030 g, 40% recovery) was purified by repeated column chromatography on silica gel (Merck Silica Gel G; particle size: 0.063 mm; ~100 times of sample weight in each case) using hexane:benzene (1:1), benzene, and benzene:ether (9:1, 4:1, 7:3, 3:2, 1:1) as eluants to give the following 10 compounds in the order of elution: **1**, **A** (=**7**), **2**, **8a**, **B** (=**6**), **C** (=**5**), **D** (=**3**), **E** (=**9**), **F** (=**4**), and **G** (=**10**).

Elemental Analysis—Satisfactory analytical data (±0.4% C,H,N) were obtained for all new metabolites and their related compounds (**1–7**, **8a**, **8b**, **9**, **10**, **12b**, **13a**, **13b**, **14–17**, **19**, **20–23**, **29a**, **29b**, **31–32**).

Spectral Data—The ^1H NMR spectra of all new metabolites and their related compounds are shown in Tables III–VI.

Identification and Characterization of Isolated Compounds—*Methyl 8,11,13,14,15-abietatetraen-18-oate (1)*—The melting point was 72–73.5 °C (from methanol); $[\alpha]_D$ +63.8° (c 0.69); IR:1720 cm^{-1}; ^1H NMR: (60 MHz).[6,17]

Dimethyl (15S)-8,11,13-abietatrien-16,18-dioate [Compound A (=7)]—The melting point was 105–106 °C (from methanol); $[\alpha]_D$ +20.9° (c 2.45); IR:1723 cm^{-1}; MS:*m/z* 358 (M^+).

Anal.—Calc. for $C_{22}H_{30}O_4$: C, H.

Conversion of Compound 7 and Methyl (15S)-16-hydroxy-8,11,13-abietatrien-18-oate (8a) into (15S)-16,18-bis(4-Nitrobenzoyloxy)-8,11,13-abietatriene (13a)—(*a*) A mixture of **7** (150 mg) and $LiAlH_4$ (32 mg) in dry ether (5.0 mL) was stirred at room temperature for 1 h. The mixture was poured into ice-dilute HCl and extracted with ether. After the usual work-up, the crude product was chromatographed on silica gel (10 g), using benzene:ether (4:1) to give (15*S*)-8,11,13-abietatrien-16,18-diol (**12a**; 109 mg; 86.1%); mp 123–126 °C (amorphous solid from acetone:hexane); $[\alpha]_D$ −54.9° (c 2.39); IR:3625 cm^{-1}. A mixture of the diol **12a** (74 mg) and 4-nitrobenzoyl chloride (136 mg) in pyridine (2.0 mL) was heated at 70–80 °C for 2.5 h. The mixture was cooled and diluted with ether. The ether solution was washed successively with dilute HCl, aqueous $NaHCO_3$, and brine. The solvent was evaporated under reduced pressure to give the residue which was chromatographed on silica gel (6 g), using benzene, to afford **13a** (128 mg: 86.9%); mp 151–152 °C (from acetone:hexane); $[\alpha]_D$ −13.1° (c 1.22); IR:1720, 1608, 1528, and 1350 cm^{-1}. The physical and spectral data of **13a** were identical with those of the authentic sample prepared as described below in (*b*).

Anal.—Calc. for $C_{34}H_{36}O_8N_2$: C, H, N.

(*b*) A mixture of **8a**[17] (34 mg) and $LiAlH_4$ (10 mg) in dry ether (2.0 mL) was stirred at room temperature for 1 h to give a crude **12a** (30 mg), which was treated with 4-nitrobenzoyl chloride (57 mg) in pyridine (2 mL) at 70–80 °C for 2.5 h. The crude product was chromatographed on silica gel (6 g), using benzene, to give *13a* (55 mg: 89%); mp 151–152 °C (from acetone:hexane); $[\alpha]_D$ −13.3° (c 1.35).

Anal.—Calc. for $C_{34}H_{36}O_8N_2$: C, H, N.

Methyl 15-hydroxy-8,11,13-abietatrien-18-oate (2) and Methyl (15S)-16-hydroxy-8,11,13-abietatrien-18-oate (8a)—Benzene:ether (9:1; Table I) eluate afforded a mixture of **2** and **8a** (941 mg) which was acetylated with acetic anhydride (5 mL) and pyridine (5 mL) at room temperature for 20 h. After the usual work-up, the crude acetate was chromatographed on silica gel (40 g) using benzene:ether (98:2) to give methyl 16-acetoxy-8,11,13-abietatrien-18-oate (432 mg). Further elution with benzene:ether (9:1) afforded **2** (179 mg): mp 77–80 °C (from hexane); $[\alpha]_D$ +51.9° (c 2.68). The physical and spectral data of **2** were identical with reported values.[30] The above 16-acetoxylated compound (432 mg) was refluxed with dilute HCl (15%, 1.5 mL) in methanol (15 mL) for 1 h. The crude product was chromatographed on silica gel (30 g), using benzene:ether (9:1), to give **8a**[6] (307 mg); $[\alpha]_D$ +48.6° (c 1.11). The IR and ^1H NMR spectra of **8a** were identical with those of authentic methyl (15*S*)-16-hydroxy-8,11,13-abietatrien-18-oate.[17]

Anal.—Calc. for $C_{21}H_{30}O_3$: C, H.

Methyl (15S)-16-benzoyloxy-8,11,13-abietatrien-18-oate (29a) and its (15R)-Epimer (29b)—(*a*) A mixture of methyl (15*S*)-16-hydroxy-8,11,13-abietatrien-18-oate (**8a**; 26 mg) and benzoyl chloride (0.02 mL) in pyridine (1.0 mL) was heated at 70–80 °C for 1.5 h. The crude product was chromatographed on silica gel (6 g), using benzene, to give oily **29a** (31.4 mg: 91.8%); $[\alpha]_D$ +29.5° (c 1.39); IR:1718 cm^{-1}.

Anal.—Calc for $C_{28}H_{34}O_4$: C, H.

(*b*) A mixture of methyl (15*R*)-16-hydroxy-8,11,13-abietatrien-18-oate (**8b**; 45 mg) and benzoyl chloride (0.03 mL) in pyridine was heated at 70–80 °C for 1.5 h. The crude product was treated in the same manner as described above to afford oily **29b** (56.4 mg: 95.3%); $[\alpha]_D$ +48.2° (c 1.54); IR:1716 cm^{-1}.

Anal.—Calc. for $C_{28}H_{34}O_4$: C, H.

Conversion of Methyl (15R)-16-hydroxy-8,11,13-abietatrien-18-oate (8b) into (15R)-16,18-bis(4-nitrobenzoyloxy)-8,11,13-abietatriene (13b)—A mixture of **8b**[17] (64 mg) and $LiAlH_4$ (25 mg) in dry ether (4.0 mL) was stirred at room temperature for 1 h. The crude product was chromatographed on silica gel (6 g), using benzene:ether (4:1) to give (15*R*)-8,11,13-abietatrien-16,18-diol (**12b**; 56.5 mg: 96.5%); mp 137.5–139 °C (from acetone:hexane); $[\alpha]_D$ +51.3° (c 2.65); IR:3625, and 3440 cm^{-1}.

Anal.—Calc. for $C_{20}H_{30}O_2$: C, H.

The diol **12b** (37 mg) was treated with 4-nitrobenzoyl chloride (68.1 mg) in pyridine (2.0 mL) at 70–80 °C for 2.5 h. The crude product was chromatographed on silica gel, using benzene to give **13b** (71.9 mg: 97.8%); mp 155–157 and 175–177 °C (from acetone:hexane); $[\alpha]_D$ +13.2° (c 2.73); IR:1720, 1610, 1530, and 1352 cm^{-1}.

Anal.—Calc. for $C_{34}H_{36}O_8N_2$: C, H, N.

Methyl 2α-hydroxy-8,11,13,15-abietatetraen-18-oate [Compound B (=6)]—The melting point was 107.5–108.5 °C (from hexane); $[\alpha]_D$ +62.5° (c 0.56); IR:3500, 3430, and 1725 cm^{-1}; MS: *m/z* 328 (M^+).

Anal.—Calc. for $C_{21}H_{28}O_3$: C, H.

Catalytic Hydrogenation of 6—A mixture of **6** (30.0 mg) and 10% Pd–C (15 mg) in ethyl acetate (5.0 mL) was hydrogenated at room temperature under an atmosphere of hydrogen for 4 h. After the usual work-up, the reaction mixture was chromatographed on silica gel (6 g), using benzene:ether (9:1), to give methyl 2α-hydroxy-8,11,13-abietatrien-18-oate (**14**; 24.4 mg: 80.8%); mp 152–154 °C (from hexane); $[\alpha]_D$ +53.6 (c 1.10); IR:3600, 3440, and 1725 cm^{-1}.

Anal.—Calc. for $C_{21}H_{30}O_3$: C, H.

A mixture of **14** (10.0 mg) and acetic anhydride-pyridine (each 0.5 mL) was allowed to stand at room temperature for 18 h. After the usual work-up, the crude acetate was chromatographed on silica gel (5 g), using benzene, to give an acetate (**15**; 10.1 mg: 89.6%): mp 82–83 °C (from petroleum ether); $[\alpha]_D$ +16.0° (c 1.25); IR:1725 cm^{-1}.

Anal.—Calc. for $C_{23}H_{32}O_4$: C, H.

Methyl (15R)-15,16-dihydroxy-8,11,13-abietatrien-18-oate [Compound C (=5)]—The melting point was 106–107 °C (from acetone:hexane); $[\alpha]_D$ +48.8° (c 5.23); IR:3575, 3425, and 1717 cm^{-1}; MS:*m/z* 346 (M^+).

Anal.—Calc. for $C_{21}H_{30}O_4$: C, H.

Conversion of 5 into Methyl (15R)-15-hydroxy-16-(4-nitrobenzoyloxy)-8,11,13-abietatrien-18-oate (16) and Methyl (15R)-16-hydroxy-8,11,13-abietatrien-18-oate (8b)—(*a*) A mixture of **5** (58.0 mg) and 4-nitrobenzoyl chloride (78.0 mg) in pyridine (2.0 mL) was allowed to stand at room temperature for 5 h. After the usual work-up, the crude product was chromatographed on silica gel (6 g), using benzene:ether (9:1), to give **16** (77.0 mg: 92.8%); mp 119–121 °C (from acetone:hexane); $[\alpha]_D$ +38.3° (c 1.41); IR:3600, 1720, 1530, and 1350 cm^{-1}.

Anal.—Calc. for $C_{28}H_{33}O_7N$: C, H, N.

(*b*) A mixture of **5** (115 mg) and methanesulfonyl chloride (0.04 mL) in pyridine (2.0 mL) was stirred at room temperature for 3 h. The mixture was poured into ice-dilute HCl and extracted with ether. The ether extract was washed successively with brine, aqueous $NaHCO_3$, and brine. The solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel (12 g), using benzene:ether (9:1), to give mesylate (**17**); 138 mg: 98.0%) as an oil; $[\alpha]_D$ +33.0 (c 1.49); IR:3600, 1700, 1360, and 1178 cm^{-1}.

Anal.—Calc. for $C_{22}H_{32}O_6S$: C, H.

A mixture of **17** (125 mg), KI (94 mg), and anhydrous K_2CO_3 (204 mg) in ethyl methyl ketone (6.0 mL) was refluxed with stirring for 4 h. The mixture was cooled, diluted with water, and extracted with ether. The ether extract was washed with brine and dried, and the solvent was evaporated to give a crude epoxide (**18**; 96.0 mg); IR:1720 cm^{-1}; ^1H NMR (60 MHz): see Tables III–VI.

A mixture of the crude 18 (96.0 mg), 10% Pd–C (50 mg), and alcoholic NaOH (2%, 0.5 mL) in ethanol (10 mL) was hydrogenated at room temperature under an atmosphere of hydrogen for 3 h. The mixture was filtered and the filtrate was acidified with acetic acid (0.06 mL). The acidic solution was evaporated under reduced pressure and the residue was extracted with ether. After the usual work-up, the crude product was chromatographed on silica gel (6 g), using benzene:ether (93:7), to give 8b (80.0 mg; 82.2% from 17); mp 71–72 °C (from hexane); $[\alpha]_D$ + 64.4° (c 1.80). The physical and spectral data of 8b were identical with those of authentic sample.[17]

Methyl 2β,15-dihydroxy-8,11,13-abietatrien-18-oate [Compound D (=3)]—The melting point was 131–132 °C (from acetone:hexane); $[\alpha]_D$ +77.7° (c 1.84); IR:3600, 3450, and 1720 cm^{-1}; MS: *m/z* 346 (M^+).

Anal.—Calc. for $C_{21}H_{30}O_4$: C, H.

Acetylation, Oxidation and Hydrogenolysis of 3—(*a*) A mixture of 3 (42.0 mg) and acetic anhydride and pyridine (each 0.5 mL) was allowed to stand at room temperature for 16 h. After the usual work-up, the crude product was chromatographed on silica gel (8 g), using benzene:ether (85:15), to give methyl 2β-acetoxy-15-hydroxy-8,11,13-abietatrien-18-oate (19; 41.0 mg: 87.1%); mp 117–118.5 °C (from hexane); $[\alpha]_D$ +70.8° (c 0.96); IR:3600, 3475, and 1725 cm^{-1}; MS:*m/z* 388 (M^+); ^1H NMR (CCl_4): see Table III.

Anal.—Calc. for $C_{23}H_{32}O_5$: C, H.

(*b*) A mixture of 3 (23.0 mg) and pyridinium chlorochromate (21.0 mg) in dichloromethane (2.0 mL) was stirred at room temperature for 1.5 h. The mixture was poured into aqueous $NaHCO_3$ and extracted with ether. The ether extract was washed with brine, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel (5 g), using benzene:ether (4:1), to give methyl 15-hydroxy-2-oxo-8,11,13-abietatrien-18-oate (20; 17.0 mg: 74.3%); mp 87–88 °C (from acetone:hexane); $[\alpha]_D$ +33.1° (c 0.82); IR:3600, 1725, and 1715 cm^{-1}.

Anal.—Calc. for $C_{21}H_{28}O_4$: C, H.

(*c*) A mixture of 3 (58.0 mg) and 10% Pd–C (120 mg) in ethyl acetate (7 mL) containing three drops of 60% perchloric acid was hydrogenated at room temperature under an atmosphere of hydrogen for 5 h. After the usual work-up, the crude product was chromatographed on silica gel (7 g), using benzene:ether (9:1), to give 2β-hydroxy-8,11,13-abietatrien-18-oate (21; 39.5 mg: 71.4%) as an oil; $[\alpha]_D$ +81.0° (c 1.66); IR:3610, 3450, and 1723 cm^{-1}.

Anal.—Calc. for $C_{21}H_{30}O_3$: C, H.

A mixture of 21 (30.0 mg) and acetic anhydride and pyridine (each 0.5 mL) was allowed to stand at room temperature for 18 h. After the usual work-up, the crude acetate was purified by column chromatography on silica gel (5 g), using benzene:ether (95:5), to give methyl 2β-acetoxy-8,11,13-abietatrien-18-oate (22; 33.3 mg: 98.5%); mp 110–111 °C (from hexane): $[\alpha]_D$ +80.5° (c 0.80); IR:1725 cm^{-1}.

Anal.—Calc. for $C_{23}H_{32}O_4$: C, H.

Methyl (15S)-2β,16-dihydroxy-8,11,13-abietatrien-18-oate [Compound E (=9)]—The melting point was 95–98 °C (amorphous solid from acetone:hexane); $[\alpha]_D$ +72.7° (c 0.83); IR:3600, 3450, and 1720 cm^{-1}; MS:*m/z* 346 (M^+).

Anal.—Calc. for $C_{21}H_{30}O_4$: C, H.

In another experiment, a mixture of 3 and 9 was acetylated with acetic anhydride and pyridine at room temperature for 16 h. The crude acetate was purified by column chromatography on silica gel to afford methyl 2β-acetoxy-15-hydroxy-8,11,13-abietatrien-18-oate (19) and methyl (15S)-2β,16-diacetoxy-8,11,13-abietatrien-18-oate (23), which were hydrolyzed with dilute HCl in methanol under reflux for 1 h to give 3 and 9, respectively.

Conversion of 9 into Methyl (15S)-16-(4-nitrobenzoyloxy)-8,11,13-abietatrien-18-oate (30)—A mixture of 9 (20.0 mg) and acetic anhydride and pyridine (each 0.5 mL) was allowed to stand at room temperature for 17 h. After the usual work-up, the crude product was chromatographed on silica gel (7 g), using benzene:ether (95:5), to give methyl (15S)-2β,16-diacetoxy-8,11,13-abietatrien-18-oate (23; 22.6 mg: 90.8%) as an oil; $[\alpha]_D$ +70.2 (c 1.13); IR:1725 cm^{-1}; MS:*m/z* 430 (M^+).

Anal.—Calc. for $C_{25}H_{34}O_6$: C, H.

A mixture of 23 (31.7 mg) and dilute HCl (15%, 0.1 mL) in methanol (1.0 mL) was stirred at 23–25 °C for 4 h. The mixture was diluted with ether, washed with brine, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel (5 g) using benzene:ether (9:1) to give the 2β-acetoxy-16-hydroxy compound (24; 23.0 mg: 80.4%).

A mixture of 24 (23.0 mg) and benzoyl chloride (0.01 mL) in pyridine (1.0 mL) was heated at 50–60 °C for 1 h. After the usual work-up, the crude product was chromatographed on silica gel (5 g) using benzene to give the 2β-acetoxy-16-benzoyloxy compound (25; 20.0 mg: 89.2%).

A mixture of 25 (20.0 mg) and dilute HCl (15%, 0.1 mL) in methanol (1.0 mL) was refluxed for 2 h. The reaction mixture was diluted with ether and, after the usual work-up, the product gave the crude 16-benzoyloxy-2β-hydroxy compound (26; 15.1 mg); IR:3600, and 1715 cm^{-1}.

A mixture of 26 (15.1 mg) and methanesulfonyl chloride (0.1 mL) in pyridine (1.0 mL) was allowed to stand at room temperature for 17 h to give a crude mesylate (27; 18.0 mg); ^1H NMR (60 MHz: see Table III–VI).

The mesylate (27; 18.0 mg) in 2,4-lutidine (1.0 mL) was refluxed for 2.5 h under a stream of N_2. The mixture was cooled, poured into dilute HCl, and extracted with ether. The ether extract was washed successively with dilute HCl, aqueous $NaHCO_3$, and brine. The solvent was evaporated and the residue was chromatographed on silica gel (5 g) using benzene to give a mixture of tetraenes (28; 9.0 mg) which was dissolved in methanol (5.0 mL) and hydrogenated in the presence of 10% Pd–C (15 mg) at room temperature under an atmosphere of hydrogen for 4 h to give the crude methyl 16-benzoyloxy-8,11,13-abietatrien-18-oate (29a; 9.0 mg). This ester was hydrolyzed with aqueous NaOH (20%, 0.1 mL) in methanol (1.0 mL) under reflux for 1 h. The mixture was diluted with ether and the ether solution was washed successively with dilute HCl, aqueous $NaHCO_3$, and brine. The solvent was evaporated under reduced pressure to give the 16-hydroxy compound which was treated with 4-nitrobenzoyl chloride (10.0 mg) in pyridine (1.0 mL) at 70–80 °C for 2 h. The crude product was chromatographed on silica gel (6 g) using benzene to give a 4-nitrobenzoate (7.0 mg); $[\alpha]_D$ +15° (c 0.29). This was identical with authentic methyl (15S)-16-(4-nitrobenzoyloxy)-8,11,13-abietatrien-18-oate (30).[17]

Methyl 2α,15-dihydroxy-8,11,13-abietatrien-18-oate [Compound F (=4)]—The melting point was 157–158.5 ° (from acetone:hexane); $[\alpha]_D$ +48.1 (c 3.29); IR:3600, 3430, and 1722 cm^{-1}; MS:*m/z* 346 (M^+).

Anal.—Calc. for $C_{21}H_{30}O_4$: C, H.

Acetylation, Oxidation and Hydrogenolysis of 4—(*a*) A mixture of 4 (23.0 mg) and acetic anhydride and pyridine (each 0.5 mL) was allowed to stand at room temperature for 17 h. The crude product was chromatographed on silica gel (5 g), using benzene:ether (4:1), to give methyl 2α-acetoxy-15-hydroxy-8,11,13-abietatrien-18-oate (31; 24.4 mg: 94.6%); mp 142–143 °C (from acetone:hexane); $[\alpha]_D$ +15.8° (c 1.14); IR:3600, 3475, and 1725 cm^{-1}.

Anal.—Calc. for $C_{23}H_{32}O_5$: C, H.

(*b*) A mixture of 4 (68.0 mg) and pyridinium chlorochromate (63.0 mg) in dichloromethane (5.0 mL) was stirred at room temperature for 1.5 h. The crude product was chromatographed on silica gel (5 g) using benzene:ether (4:1) to give a hydroxy keto ester (46.0 mg: 68.0%); mp 87–88 °C (from acetone:hexane); $[\alpha]_D$ +33.4° (c 2.12). The physical and spectral data of the hydroxy keto ester were identical with those of 20 prepared from compound D (=3).

(*c*) A mixture of 4 (28.0 mg) and 10% Pd–C (50 mg) in ethyl acetate (5.0 mL) containing three drops of 60% perchloric acid was hydrogenated at room temperature under an atmosphere of hydrogen for 4 h. The crude product was chromatographed on silica gel (5 g) using benzene:ether (4:1) to give methyl 2α-hydroxy-8,11,13-abietatrien-18-oate (19.1 mg: 71.5%), which was identical with authentic sample (14) prepared from compound B (=6).

Methyl (15S)-2α,16-dihydroxy-8,11,13-abietatrien-18-oate [Compound G (=10)]—The melting point was 147–148 °C (from acetone:hexane); $[\alpha]_D$ +53.9° (c 0.58); IR:3600, 3430, and 1722 cm^{-1}; MS:*m/z* 346 (M^+).

Anal.—Calc. for $C_{21}H_{30}O_4$: C, H.

In another experiment, a mixture of 4 and 10 was acetylated with acetic anhydride in pyridine at room temperature for 16 h. The crude product was purified by column chromatography on silica gel to give methyl 2α-acetoxyl-5-hydroxy-8,11,13-abietatrien-18-oate (31) and methyl (15S)-2α,16-diacetoxy-8,11,13-abietatrien-18-oate (32) which were hydrolyzed with dilute HCl in methanol under reflex for 1 h to give 4 and 10, respectively.

Conversion of 10 into Methyl (15S)-16-benzoyloxy-8,11,13-abietatrien-18-oate (29a)—A mixture of 10 (61.0 mg) and acetic anhydride (0.5 mL) in pyridine (0.5 mL) was allowed to stand at room temperature for 17 h. The crude product was chromatographed on silica gel (7 g) using benzene:ether (95:5) to afford methyl (15S)-

2α,16-diacetoxy-8,11,13-abietatrien-18-oate (**32**; 69.0 mg: 91.0%) as an oil; $[\alpha]_D$ +13.8 (c 1.45); IR:1725 cm^{-1}; MS:*m/z* 430 (M^+).

Anal.—Calc. for $C_{25}H_{34}O_6$: C, H.

A mixture of **32** (58.9 mg) and dilute HCl (15%, 0.7 mL) in methanol (2.0 mL) was stirred at 23–25 °C for 2 h. The crude product was chromatographed on silica gel (10 g) using benzene:ether (95:5) to give the starting diacetate **32** (19.1 mg). Subsequent elution with benzene:ether (4:1) afforded the 2α-acetoxy-16-hydroxy compound (**33**; 25.9 mg; 48.7%); IR:3600, 3450, and 1725 cm^{-1}. Further elution with ether afforded the 2α,16-dihydroxy compound (**10**; 6.5 mg: 14%).

A mixture of **33** (27.0 mg) and benzoyl chloride (0.01 mL) in pyridine (1.0 mL) was heated at 50–60 °C for 2 h. The crude product was chromatographed on silica gel (6 g) using benzene:ether (9:1) to give the 2α-acetoxy-16-benzoyloxy compound (**34**; 33.2 mg: 97.0%).

A mixture of **34** (41.0 mg) and dilute HCl (15%, 0.15 mL) in methanol (1.5 mL) was refluxed for 1 h to give the crude 16-benzoyloxy-2-hydroxy compound (**35**; 37.0 mg); IR:3600, 3440, and 1720 cm^{-1}.

A mixture of **35** (37.0 mg) and methanesulfonyl chloride (0.04 mL) in pyridine (1.0 mL) was heated at 50–60 °C for 1 h to give a crude mesylate (**36**; 45.0 mg); ^1H NMR (60 MHz: see Tables III–VI).

A solution of **36** (45.0 mg) in 2,4-lutidine (1.0 mL) was refluxed for 7 h under a stream of N_2. The crude product was chromatographed on silica gel (5 g) using benzene to give a mixture of tetraenes (**28**; 18.7 mg) which was hydrogenated in methanol (5.0 mL) in the presence of 10% Pd–C (25 mg) at room temperature for 4 h. The crude product was chromatographed on silica gel (7 g) using benzene to give an oily benzoate (10.7 mg); $[\alpha]_D$ +30.5° (c 0.41). This benzoate was identical with authentic methyl (15*S*)-16-benzoyloxy-8,11,13-abietatrien-18-oate (**29a**).[17]

References and Notes

1. Ishida, T.; Asakawa, Y.; Okano, M.; Aratani, T. *Tetrahedron Letters* **1977**, 2437–2440.
2. Ishida, T.; Asakawa, Y.; Takemoto, T.; Aratani, T. *J. Pharm. Sci.* **1979**, *68*, 928–930.
3. Ishida, T.; Asakawa, Y.; Takemoto, T.; Aratani, T. *J. Pharm. Sci.* **1981**, *70*, 406–415.
4. Asakawa, Y.; Taira, Z.; Takemoto, T.; Ishida, T.; Kido, M.; Ichikawa, Y. *J. Pharm. Sci.* **1971**, *70*, 710–711.
5. Ishida, T.; Asakawa, Y.; Takemoto, T. *J. Pharm. Sci.* **1982**, *71*, 965–966.
6. Asakawa, Y.; Ishida, T.; Toyota, M.; Takemoto, T. *Xenobiotica* **1986**, *16*, 753–767.
7. Asakawa, Y.; Toyota, M.; Ishida, T. *Xenobiotica* **1988**, *18*, 1129–1134.
8. Ishida, T.; Toyota, M.; Asakawa, Y. *Xenobiotica* **1989**, *19*, 843–855.
9. Mueller, J. C.; Learch, J. M.; Walden, C. C. *Tappi* **1977**, *60*, 135–137.
10. Mueller, J. C.; Learch, J. M.; Walden, C. C. *Tappi Environ. Conf. Prepr.* 77–81; *Chem. Abstr.* **1977**, *87*, 90194q.
11. Learch, J. M.; Mueller, J. C.; Walden, C. C. *Prep. Paper Ann. Meet. Tech. Sect., C.P.P.A.*, 63rd, **1977**, A, 135–140; *Chem. Abstr.* **1977**, *89*, 151985p.
12. Mattsoff, L.; Nikinmaa, M. *Ecotoxicol. Environ. Saf.* **1987**, *14*, 157–163; *Chem. Abstr.* **1988**, *108*, 50778d.
13. Kutney, J. P.; Singh, M.; Hewitt, G.; Salisburry, P. J.; Worth, B. R.; Servizi, J. A.; Martens, D. W.; Gordon, R. W. *Can. J. Chem.* **1981**, *59*, 2334–2341.
14. Kieslich, K. *Microbial Transformation of Non-Steroid Cyclic Compounds;* George Thieme: Stuttgart, **1976**; pp 77–80, 342–1105.
15. Ekman, R.; Sjoholm, R. *Acta Chemica Scand. B* **1979**, *33*, 76–78.
16. Sato, Y.; Suzuki, Y.; Shibata, M. *Shokuhin Kogyo* **1981**, *24*, 57–62.
17. Matsumoto, T.; Imai, S.; Hayashi, N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2405–2411.
18. Bang, Luu; Ourisson, G. *Tetrahedron Lett.* **1975**, 1881–1884.
19. Wadel, A.; Ve, B.; Scheline, R. R.; Monge, P. *Xenobiotica* **1983**, *13*, 503–512.
20. Buset, H.; Scheline, R. R. *Biomedical Mass Spectrometry* **1979**, *6*, 212–220.
21. Kodama, R.; Noda, K.; Ide, H. *Xenobiotica* **1974**, *4*, 85–95.
22. Ide, H.; Toki, S.; *Biochem. J.* **1970**, *119*, 281–287.
23. Mitsui, S., Imaizumi, S. *J. Chem. Soc. Jpn. (Nippon Kagaku Zasshi)* **1965**, *86*, 219–224.
24. Bakke, O. M.; Scheline, R. R. *Toxicol. Pharmacol.* **1970**, *16*, 691–700.
25. Sugiyama, K.; Trager, W. *Biochemistry* **1986**, *25*, 7336–7343.
26. Goeneckea, S.; Olek, K.; Wardenbach, P. *J. Chromatogr.* **1978**, *154*, 282–284.
27. Sullivan, H. R.; MacMahon, R. E. *Mass Spectrum Metab. (Proc. Int. Symp.)*; Albert, F., Ed.; **1976**; (Pub. 1977), pp 31–43.
28. Mash, E. A.; Math, S. K. *Toxicol. Environ. Chem.* **1988**, *17*, 197–213.
29. Biellmann, J. F.; Branlant, G. *Tetrahedron* **1973**, *29*, 1227–1236.
30. Norin, T.; Winell, B. *Acta Chem. Scand.* **1972**, *26*, 2289–2296.

Acknowledgments

The authors are grateful to Arakawa Chemical Industry, Company, Ltd. for the donation of (+)-dehydroabietic acid. One of the authors (T.I.) thanks Prof. G. Bramlamt, Universite Louis Pasteur de Strasbourg, for the kind sending of various kinds of derivatives of dehydroabietic acid and Prof. J. P. Kutney, University of British Columbia for his generous gift of mass spectra of metabolites of dehydroabietic acid by the micro-organism *Mortierella*. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare (Y.A.).