SANDARAC ACIDS: 6β-HYDROXYSANDARACOPIMARIC ACID[1]

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ABSTRACT

The resin from *Callitris quadrivalvis* Vent. Decad. (Sandarac resin) has been shown to contain 6β-hydroxysandaracopimaric acid (6β-hydroxy-7-epipimara-9(14),19-dienoic acid).

Recently we reported the isolation of what appeared to be an hydroxypimaric acid from commercial Sandarac resin (1) derived from the North African tree *Callitris quadrivalvis* Vent. Decad. We now present proof that this is the 6β-hydroxy derivative of sandaracopimaric acid, the structure and stereochemistry of which has been established (1, 2, 3).

The acid, m.p. 269–270°, analyzed correctly for $C_{20}H_{30}O_3$. Characteristic bands for a vinyl group at 904 and 987 cm^{-1} were present in its infrared spectrum. These were absent in the dihydro acid produced by rapid uptake of 1 mole of hydrogen over palladium. The dihydro acid had absorption at 823 cm^{-1}, suggestive of the presence of a trisubstituted double bond. The presence of this feature was confirmed by one-hydrogen signals near $\tau = 5$ (CCl_4) in the N.M.R. spectrum of a number of derivatives of the acid.

The infrared spectrum of the original acid and the dihydro acid had peaks near 3400 cm^{-1} which were absent in their readily prepared monoacetates. Thus all the functions of a hydroxypimaric acid had been identified. The hydroxyl group was shown to be secondary by oxidation of the dihydro acid to a keto acid. The structure and stereochemistry of the skeleton was then established by Wolff–Kishner reduction of the keto acid. The product was identical in all respects with dihydrosandaracopimaric acid (I) (1).

The N.M.R. spectrum (CCl_4) of the methyl ester of the keto dihydro acid had a one-hydrogen signal at $\tau = 4.76$ (vinyl hydrogen) and what appeared to be a fairly sharp two-hydrogen signal (half band width 5 c.p.s.) at $\tau = 7.66$ superimposed on a broad unresolved signal. This had to be associated with a methylene adjacent to the ketone carbonyl. The only locations for the carbonyl which seemed compatible with the apparent lack of coupling of this methylene group with other hydrogens were C-5 and C-10. However, the low field signal was absent for the 13-hydrogen expected if the carbonyl was on C-5, and the methylene signal was at too high field for one flanked by a 10-carbonyl and the double bond (expected around $\tau = 7$). In addition, neither base nor acid treatment of the keto acid moved the double bond into conjugation with the carbonyl. Hence neither of these positions for the oxygen appeared likely.

In order to establish the oxygen location the dihydro keto acid was treated with methyl magnesium iodide. The resulting hydroxy acid was dehydrogenated using selenium. The major product was 1,6,7-trimethylphenanthrene (II), thus proving that the keto acid was 6-keto-19,20-dihydrosandaracopimaric acid (III).

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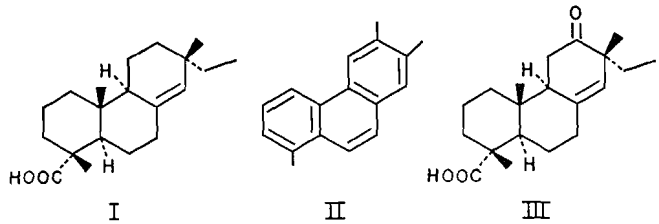


I II III

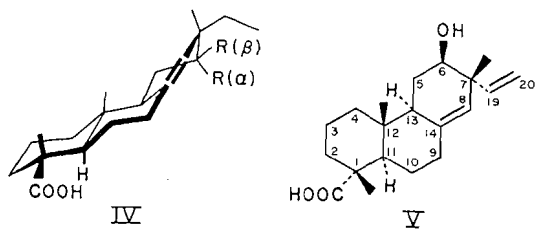
In retrospect, an important clue about the location of the oxygen could have been the chemical shift on the 18-methyl signal in the 6-ketone, as compared with that in the other derivatives (Table I). A comparable shift for the C-18 in 17a-keto-D-homosteroids has been recorded (4).

TABLE I

Chemical shifts in τ in carbon tetrachloride ($Si(CH_3)_4$ as internal standard)

	C_{16}	C_{18}	C_{17}	Vinyl proton	COOH	$COOCH_3$
Methyl dihydrosandaracopimarate	8.89	9.18	9.26	5.02	—	6.48
Methyl dihydro-6-hydroxysandaracopimarate	8.87	9.16	9.21	5.11	—	6.53
Methyl dihydro-6-ketosandaracopimarate	8.89	9.00	9.27	4.76	—	6.38
Dihydro 6-acetoxysandaracopimaric acid	8.88	9.11	9.22	5.18	−1.14	—

There remained the problem of assigning configuration to the hydroxyl group of the original acid. Since the epimeric hydroxyls on C-6 should be true axial and true equatorial in the half-chair conformation of ring C (see IV) we chose to examine the products of borohydride and sodium in alcohol reduction of the 6-ketone. In both cases, high yields of product with the original hydroxyl configuration were formed. This established the hydroxyl conformation as equatorial. Thus the hydroxyl in the original acid is β oriented (see IV) and the new acid is 6β-hydroxysandaracopimaric acid (V) (6β-hydroxy-7-epipimara-8(14),19-dienoic acid).

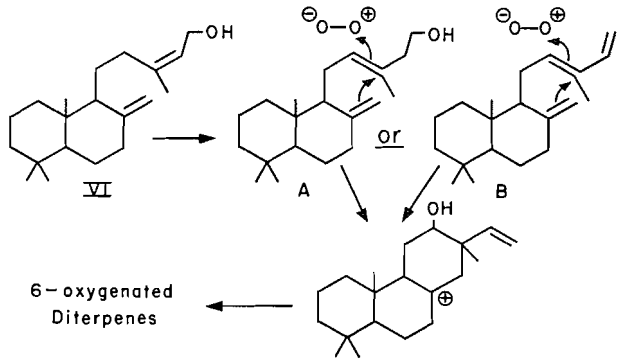


IV V

This acid takes its place with podocarpic acid, ferruginol, Δ^9-dehydroferruginol, xanthoperol, sugiol (5), nimbiol (6), royleanone (7), hinokiol and hinokione (8), vinhaticoic acid (9), and possibly the tanshinones (10) as a 6-oxygenated diterpene. The occurrence of numerous 6-oxygenated diterpenes leads us to suggest that the cyclization to form ring C of the tricyclic diterpenes can take place not only from VI or manool (11) but also in some plants from the double bond isomer A or triene B.[3] Proton-initiated cyclization of these would lead to hydroxyl-free molecules, but cyclization initiated by oxygen[4] would lead to the 6-oxygenated diterpenes listed above.

[3]*This triene B has recently been isolated from Dacrydium biforme and named biformene (12).*

[4]*The work of Tchen and Bloch (13) established the initiation of squalene cyclization in yeast to give 3-hydroxy steroids to be by atmospheric oxygen, presumably enzyme activated.*



EXPERIMENTAL

Rotations were of ethanol solutions, infrared spectra were of nujol mulls, and melting points were taken on a Kofler hot stage.

6-Hydroxysandaracopimaric Acid

The acid was obtained as described previously (1). It crystallized from acetone as colorless needles, m.p. 269–270°, $[\alpha]_D$ −11° (*c*, 0.4). Its infrared spectrum had weak bands at 904 and 987 cm^{-1} (vinyl group) and 822 cm^{-1} ($\rangle C{=}C\langle^{H}$), and a strong band at 3360 cm^{-1} (hydroxyl) and at 1695 cm^{-1} (—COOH).

6-Hydroxy-19,20-dihydrosandaracopimaric Acid

6-Hydroxysandaracopimaric acid (480 mg) in ethanol (250 ml) was reduced with hydrogen in the presence of presaturated 10% palladium on charcoal (400 mg). In 3 minutes, 36 ml of hydrogen were absorbed (calc. for 1 mole 35 ml). The product crystallized from methanol or ethanol as colorless plates (450 mg), m.p. 294–295°, $[\alpha]_D$ +17.5 (*c*, 0.28). Found: C, 75.12; H, 9.98. Calc. for $C_{20}H_{32}O_3$: C, 74.96; H, 10.06%. Infrared spectrum: ν_{max} 3360 cm^{-1} (hydroxyl); 1695 cm^{-1} (—COOH); 823 cm^{-1} ($\rangle C{=}C\langle^{H}$).

6-Acetoxysandaracopimaric Acid

6-Hydroxysandaracopimaric acid (200 mg), pyridine (2 ml), and acetic anhydride (2 ml) were allowed to stand overnight at room temperature. The colorless solution was poured into water and the solid product crystallized from *n*-hexane. Recrystallization from the same solvent gave colorless prisms (200 mg), m.p. 170°, $[\alpha]_D$ −51° (*c*, 1.56). Found: C, 73.41; H, 8.79. Calc. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Infrared spectrum: ν_{max} 1730 cm^{-1} (acetate); 1700 cm^{-1} (—COOH); 909 cm^{-1} and 992 cm^{-1} (vinyl group); and 826 cm^{-1} ($\rangle C{=}C\langle^{H}$).

6-Acetoxy-19,20-dihydrosandaracopimaric Acid

Treatment of the dihydro acid (100 mg) in the manner described above yielded colorless prisms (90 mg), m.p. 183°, from *n*-hexane. $[\alpha]_D$ −27.5° (*c*, 0.08). Found: C, 73.09; H, 9.21. Calc. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Infrared spectrum: ν_{max} 1725 cm^{-1}, 1700 cm^{-1} (—COOH).

Methyl 6-Hydroxysandaracopimarate

6-Hydroxysandaracopimaric acid (50 mg) in methanol (25 ml) was treated with excess ethereal diazomethane during 15 minutes. Excess diazomethane was destroyed with acetic acid. The solution was washed well with sodium bicarbonate and water; drying and evaporation yielded a crystalline residue (50 mg). Recrystallization from *n*-hexane gave colorless needles (45 mg), m.p. 94°. The analytical sample was sublimed at 80°/10^{-3} mm. Found: C, 76.02; H, 9.61; OMe, 9.12. Calc. for $C_{20}H_{29}O_2(OMe)$: C, 75.86; H, 9.70; OMe, 9.34. Infrared spectrum: ν_{max} 3300 cm^{-1} (hydroxyl), 1730 cm^{-1} (—COOMe), 909 and 992 cm^{-1} (vinyl group), and 827 cm^{-1} ( ).

Methyl 6-Hydroxy-19,20-dihydrosandaracopimarate

The dihydro acid (30 mg) on treatment with diazomethane in the manner described above yielded colorless needles (27 mg), m.p. 112°, from *n*-hexane. Found: C, 75.20; H, 10.06; OMe, 9.04. Calc. for $C_{20}H_{31}O_2(OMe)$: C, 75.40; H, 10.25; OMe, 9.25. Infrared spectrum: ν_{max} 3360 cm^{-1} (hydroxyl), 1730 cm^{-1} (—COOMe), and 828 cm^{-1} ().

6-Keto-19,20-dihydrosandaracopimaric Acid

6-Hydroxy-19,20-dihydrosandaracopimaric acid (570 mg) in benzene (50 ml) and acetic acid (50 ml) was treated with sodium dichromate dihydrate (200 mg) in acetic acid (10 ml) at room temperature. Next day the green reaction mixture was poured into water and the benzene layer separated. The aqueous layer was further extracted with benzene (4×25 ml). The combined benzene extracts were dried and evaporated, yielding a crystalline residue (470 mg). Recrystallization from ether/*n*-hexane in an acetone – dry ice bath gave colorless prisms, m.p. 149°. The analytical sample was sublimed at 120°/10^{-3} mm. $[\alpha]_D$ −79° (*c*, 0.38). Found: C, 75.43; H, 9.61. Calc. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Infrared spectrum: ν_{max} 1705 cm^{-1} ( ), 1690 cm^{-1} (—COOH).

Methyl 6-Keto-19,20-dihydrosandaracopimarate

The above keto acid (110 mg) in ether (10 ml) and methanol (5 ml) was treated with excess ethereal diazomethane during 15 minutes. Excess diazomethane was decomposed with acetic acid and the ethereal solution washed with sodium bicarbonate and water. The dried solution on evaporation yielded a colorless crystalline product (112 mg), m.p. 88–89°. On recrystallization from pentane and sublimation, this gave prisms, m.p. 89°. $[\alpha]_D$ −71° (*c*, 1.4). Found: C, 75.77; H, 9.53; OMe, 9.22. Calc. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70; OMe, 9.34. Infrared spectrum: ν_{max} 1714 cm^{-1} (—COOMe), 1706 cm^{-1} (6-membered ketone).

Wolff–Kishner Reduction of 6-Keto-19,20-dihydrosandaracopimaric Acid

6-Keto-19,20-dihydrosandaracopimaric acid (58 mg) in hydrazine hydrate (2 ml) and triethylene glycol (2 ml) was heated to 140° during 45 minutes. Potassium hydroxide (1 g) was added during 5 minutes and the temperature gradually raised to 200° for 5 hours. The reaction mixture was cooled, acidified with 2 *N* H_2SO_4, and ether extracted. The ether extract was washed well with water, dried, and evaporated, yielding a colorless crystalline solid (43 mg), m.p. 175–179°. Recrystallization from aqueous methanol gave needles, m.p. 177–179° undepressed on mixture with dihydro sandaracopimaric acid, and having the requisite infrared spectrum, $[\alpha]_D$ +25° (*c*, 1.25). Found: C, 78.76; H, 10.62. Calc. for $C_{20}H_{32}O_2$: C, 78.94; H, 10.52.

Reaction of Methyl Magnesium Iodide with 6-Ketodihydrosandaracopimaric Acid

The keto acid (1.54 g) in dry ether (20 ml) was added gradually to methyl magnesium iodide at 0°, prepared from magnesium (0.75 g) and methyl iodide (5 ml) in ether (20 ml). A colorless solid was precipitated. The mixture was vigorously stirred overnight at room temperature. It was poured onto a mixture of ice (20 mg) and 2 *N* H_2SO_4 (50 ml), then extracted with ether. The ether extract was washed well with sodium bisulphite and water. Drying and evaporation gave a colorless semicrystalline product (1.3 g). Crystallization from chloroform/*n*-hexane yielded small colorless prisms (750 mg), m.p. 218°, $[\alpha]_D$ +63° (*c*, 0.4). Found: C, 75.30; H, 10.06. Calc. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Infrared spectrum ν_{max} at 3500 cm^{-1} (OH), 1700 cm^{-1} (—COOH).

Selenium Dehydrogenation of 6-Hydroxy-6-methyl-19,20-dihydrosandaracopimaric Acid

The Grignard product (600 mg) and selenium (2 g) were intimately mixed and heated at 300° C for 17 hours. Hydrogen selenide production was followed on a tower of bleaching powder. The mixture was cooled and the black solid was extracted several times with boiling benzene. The benzene extract was refluxed with freshly precipitated silver for 1 hour, filtered, and refluxed again with fresh silver. Drying and evaporation yielded yellow prisms (350 mg), m.p. 105–120°. Chromatography on Grade IV alumina in pentane gave colorless prisms (290 mg), m.p. 110–117°. Recrystallization from aqueous ethanol gave colorless needles, m.p. 122–123°. Sublimation at $120/10^{-3}$ mm gave colorless prisms, m.p. 123°. Found: C, 92.52; H, 7.20. Calc. for $C_{17}H_{16}$: C, 92.68; H, 7.32. Its ultraviolet spectrum was identical in all respects with that reported for 1,6,7-trimethyl phenanthrene (14). Its picrate separated as orange needles from ethanol, m.p. 167–168° (reported m.p.167° (15)). Found: C, 61.34; H, 4.39. Calc. for $C_{23}H_{19}O_7N_3$: C, 61.47; H, 4.26. Its styphnate crystallized from acetic acid as orange needles. Sublimation gave the analytical sample, m.p. 171° (reported 111°, error?). Found: C, 59.22; H, 3.91. Calc. for $C_{23}H_{19}O_8N_3$: C, 59.35; H, 4.12.

The phenanthrene (91.5 mg) in acetic acid (5 ml) was oxidized with chromic trioxide (100 mg, 2.2 mole) in acetic acid (5 ml) on a steam bath for 2 hours. The reaction mixture was diluted and extracted with ether. The ether solution was washed with potassium carbonate solution and water. It was then dried and evaporated, yielding an orange crystalline product (80 mg), m.p. 215–220°. Sublimation gave orange prisms of 1,6,7-trimethyl phenanthraquinone, m.p. 223° (reported m.p. 222° (15)). The quinoxaline of the phenanthraquinone formed buff needles from aqueous ethanol, m.p. 188–189° (reported m.p. 189–190° (15)).

Reduction of the Keto Acid

6-Keto-19,20-dihydrosandaracopimaric acid (200 mg) in 80% methanol (30 ml) was treated with sodium borohydride (150 mg) and allowed to stand at room temperature for 1 hour. The mixture was acidified with 2 *N* hydrochloric acid, methanol removed *in vacuo*, and ether extracted. The ether extract was dried and evaporated, yielding a yellow oil (200 mg) which was dissolved in methanol, refluxed with charcoal, filtered, and concentrated (5 ml). On cooling, colorless needles formed (105 mg), m.p. 294–295°, $[\alpha]_D$ +17° (*c*, 0.24). Its infrared spectrum was identical with that of dihydro-6-hydroxy sandaracopimaric acid and a mixed melting point was undepressed. Sodium in alcohol reduction of the keto acid (100 mg) afforded the same product (72 mg), m.p. and mixed m.p. 295°, $[\alpha]_D$ +19° (*c*, 0.34), and having the requisite infrared spectrum.

Attempted Isomerization of the Keto Acid

Hydrogen chloride was passed into a solution of 6-keto-19,20-dihydrosandaracopimaric

acid (30 mg) in dry chloroform (10 ml) at −5° for 4 hours. The chloroform solution was washed free from mineral acid with water, and then dried and evaporated to give a colorless crystalline product (28 mg), m.p. and mixed with starting material 149° and having the requisite infrared spectrum. The keto acid was unchanged by warm 5% sodium hydroxide or by traces of perchloric acid. Similar results were observed with the dihydro hydroxy acid and the dihydro acetoxy acid.

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