

The acidic part was removed with dilute  $\text{Na}_2\text{CO}_3$  solution (2%). The neutral part (500 mg) showed IR absorptions at 1760 and  $1740\text{ cm}^{-1}$ . It was chromatographed over neutral alumina (18 g). Benzene-ether (50:1) eluted the  $\gamma$ -lactone XVI (360 mg) as a liquid, which was evaporatively distilled at  $130^\circ\text{C}$  (0.1 mmHg):  $\nu_{\text{max}}$  3620,  $1760\text{ cm}^{-1}$ ; NMR  $\delta$  0.9 (3 H, d,  $J = 7\text{ Hz}$ ), 1.3 (3 H, s), and 2.2 (1 H, s, exchangeable);  $M^+$   $m/e$  212. Anal. Calcd for  $\text{C}_{12}\text{H}_{20}\text{O}_3$ : C, 67.9; H, 9.5. Found: C, 67.5; H, 9.4. Elution with benzene-ether (1:1) afforded the solid  $\delta$ -lactone XXI (20 mg): mp  $150^\circ\text{C}$  (EtOAc-light petroleum);  $\nu_{\text{max}}$  3620,  $1740\text{ cm}^{-1}$ ; NMR  $\delta$  0.95 (3 H, d,  $J = 7\text{ Hz}$ ), 1.34 (3 H, s), and 2.2 (1 H, s, exchangeable). Anal. Calcd for  $\text{C}_{12}\text{H}_{20}\text{O}_3$ : C, 67.9; H, 9.4. Found: C, 67.6; H, 9.4.

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**Registry No.**—V, 61426-32-2; VI, 61426-33-3; VII, 61426-34-4; VIII, 61475-98-7; IX, 61426-35-5; Xa, 19923-89-8; Xb, 61426-36-6; XIa, 61426-37-7; XIb, 61426-38-8; XIIa, 61426-39-9; XIIb, 61426-40-2; XIIIa, 61426-41-3; XIIIb, 61426-42-4; XIV, 19946-75-9; XVa, 61426-43-5; IVb, 61426-44-6; XVI, 61426-45-7; XX, 61475-99-8; XXI, 61527-74-0; 2,7-dimethylcycloheptanone isomer a, 21631-95-8; 2,7-dimethylcycloheptanone isomer b, 21631-93-6; *cis*-2,7-dimethylcycloheptanone 2,4-dinitrophenylhydrazone, 21631-96-9; *trans*-2,7-dimethylcycloheptanone 2,4-dinitrophenylhydrazone, 21631-94-7; 2-methyl-2-ethoxycarbonylcycloheptanone, 20043-64-5; diethyl  $\alpha$ -

methylsuberate, 61426-46-8; 2-ethoxycarbonyl-2,7-dimethylcycloheptanone, 7272-18-6; allyl bromide, 106-95-6;  $\text{Ac}_2\text{O}$ , 108-24-7;  $\text{CH}_2\text{N}_2$ , 624-90-8.

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## The Structure of Benulin, a New Pentacyclic Triterpene Hemiketal Isolated from *Bursera arida* (Burseraceae)

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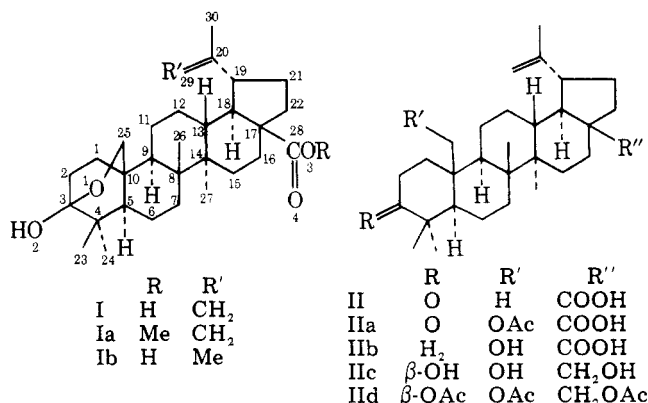
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Investigation of the chloroform extract of *Bursera arida* (Burseraceae) resulted in the isolation of a new pentacyclic triterpene hemiketal which was named benulin. On the basis of biogenetic considerations and physical and chemical data, benulin was postulated to be  $3\alpha$ -hydroxy-3,25-epoxylup-20(29)-en-28-oic acid. This structure was confirmed by an x-ray study of benulin.

Fractionation of the chloroform extract of the stems, leaves, twigs, and bark of *Bursera arida* (Rose) Standl (Burseraceae)<sup>2</sup> yielded, in addition to  $\beta$ -sitosterol, naringenin, betulonic acid, and four new lignans, benulin, a new pentacyclic triterpene hemiketal. Benulin (I) is  $3\alpha$ -hydroxy-3,25-epoxylup-20(29)-en-28-oic acid.

### Results and Discussion

Elemental analysis and molecular weight determination suggested the molecular formula  $\text{C}_{30}\text{H}_{46}\text{O}_4$  for benulin. The general appearance of the IR and NMR spectra and the fragmentation pattern in the mass spectrum suggested a triterpene with a lupane skeleton. Spectral data (IR, NMR, mass) indicated the presence of a carboxyl and an isopropenyl group in benulin. Confirmation was established by the preparation of the methyl ester (Ia) and the dihydro (Ib) derivatives and their spectral data.



The presence of a hydroxyl group and hemiketal linkage was established by the acetylation of benulin, leading to keto

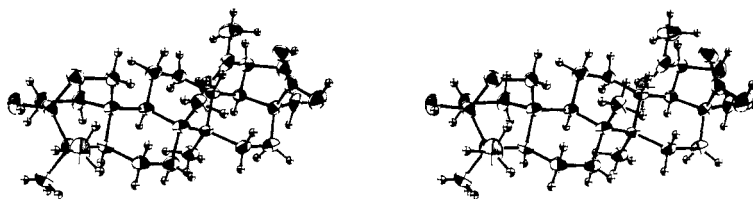


Figure 1. Stereoscopic view of a single molecule. Hydrogen atoms are shown as spheres, and other atoms as 50% probability ellipsoids.

monoacetate IIa. The IR spectrum displayed, in addition to a carboxyl carbonyl band ( $1690\text{ cm}^{-1}$ ) and an acetate carbonyl band ( $1740\text{ cm}^{-1}$ ), a third band at  $1710\text{ cm}^{-1}$  (cyclohexanone). This evidence indicated the presence of a hemiketal linkage which, under acetylating conditions, cleaved to generate a cyclohexanone system with a hydroxymethylene group which was subsequently acetylated. Spectral data (mass, NMR) confirmed the presence of a  $-\text{CH}_2\text{OAc}$  group in IIa. The mass spectrum of its Huang-Minlon reduction product IIb (loss of  $14 + 42$  mass units) and a deuterated product (gain of 2 mass units) indicated clearly the presence of a carbonyl group in the acetylated product. Basic hydrolysis of the acetate produced the parent compound. In view of its resistance toward acetylation, the hydroxyl group involved in the hemiketal linkage of I is presumably tertiary. Lithium aluminum hydride reduction of methyl benulinatate (Ia) not only reduced the ester group but also ruptured the hemiketal linkage to give a triol (IIc) which on acetylation furnished a triacetate (IIId).

The possible site for the hemiketal linkage was established by the mass spectrum fragmentation pattern of benulin and its derivatives. The most noteworthy peak in the mass spectrum of benulin is at  $m/e$  313. Esterification or hydrogenation of benulin exerted no influence on this peak, nor on the general fragmentation pattern. Acetylation, however, which disturbed the hemiketal linkage, not only wiped out the peak at  $m/e$  313 but also altered the fragmentation pattern. The pronounced loss of  $M - \text{AcOH}$  and  $M - \text{CH}_2\text{OH}$  in the mass spectrum of monoacetate IIa and its Huang-Minlon reduction product (IIb), respectively, indicated that the hemiketal linkage originated at an angular position. The co-occurrence of benulin with betulonic acid, a triterpene known to have structure II, in the same plant led to I as the most biogenetically satisfactory structure for benulin.

An x-ray study was successfully carried out on crystalline benulin. As can be seen from the resulting ORTEP<sup>3</sup> plot in Figure 1, the structure is indeed I. Rings B, C, and D have chair conformations, as in  $\beta$ -acetoxy-20-hydroxylupane,<sup>4</sup> the other lupane type triterpenoid whose structure has been studied by x-ray diffraction. Ring A and the other rings of the oxabicyclo[2.2.2]octane system have boat conformations. Ring E has the envelope conformation as evidenced by torsion angles, starting from C17-C18-C19-C21 and going clockwise, of  $21.5$ ,  $4.2$ ,  $-28.7$ ,  $41.3$ , and  $-38.9^\circ$ . The torsion angles C18-C19-C20-C29 and C18-C19-C20-C30 are  $-71.1$  and  $-11.1^\circ$ , respectively. The torsion angles around C17-C28 are C18-C17-C28-O3 ( $162.9^\circ$ ), C18-C17-C28-O4 ( $-16.8^\circ$ ), C22-C17-C28-O3 ( $-86.5^\circ$ ), and C22-C17-C28-O4 ( $93.6^\circ$ ).

The average C-C distances in rings A, B, C, D, and E are  $1.554$ ,  $1.557$ ,  $1.558$ ,  $1.560$ , and  $1.554\text{ \AA}$ , and the corresponding bond angle values are  $109.5$ ,  $111.9$ ,  $110.0$ ,  $110.7$ , and  $104.4^\circ$ , respectively. Only C9-C10 ( $1.598\text{ \AA}$ ) and C8-C14 ( $1.599\text{ \AA}$ ), between highly substituted carbons, are significantly longer. The molecular packing is governed partly by intermolecular hydrogen bonds between O2-O4 ( $2.727\text{ \AA}$ ) and O3-O1 ( $2.717\text{ \AA}$ ), which bind molecules infinitely in the  $a$  direction. The only other intermolecular distances less than  $3.5\text{ \AA}$  are O1-O4 ( $3.414\text{ \AA}$ ) and O2-C28 ( $3.467\text{ \AA}$ ).

Benulin (I) has not previously been synthesized, nor isolated from a natural source. Therefore it is the first triterpene with

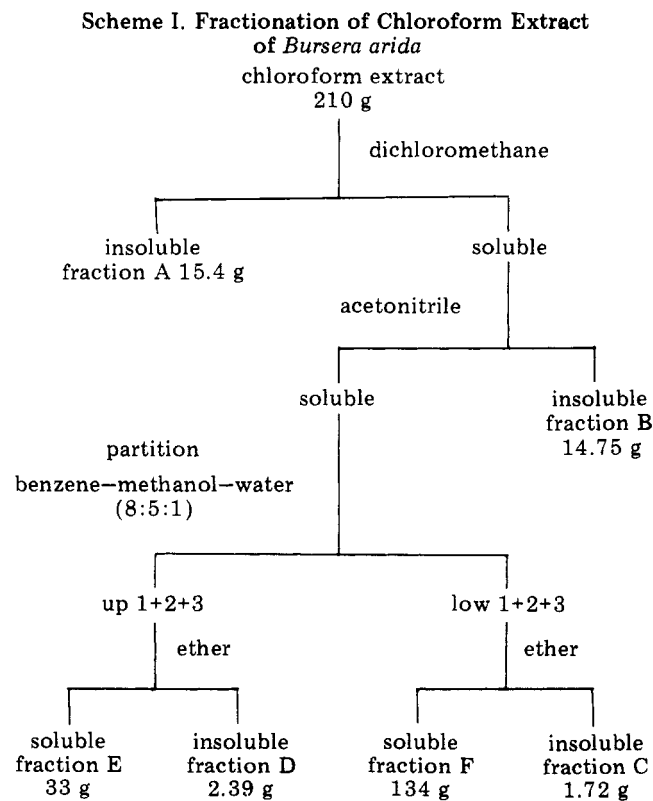
a lupane skeleton for which a hemiketal linkage terminating at a C-3 bearing hydroxyl group has been established. Biosynthetically, it may arise from betulonic acid (II), which accompanies it in *Bursera arida*, by hydroxylation at C25 followed by hemiketal formation.

### Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Carbon and hydrogen analyses were carried out by Chemalytics, Inc., Tempe, Ariz. Infrared (IR) spectra were run on a Beckman IR-33 spectrophotometer. Unless otherwise mentioned all nuclear magnetic resonance (NMR) spectra were run in  $\text{CDCl}_3$  using Varian T-60 and HA-100 instruments and peak positions are given in  $\delta$  values, using tetramethylsilane as an internal standard. Mass spectra were recorded on a Hitachi Perkin-Elmer Model RMU-6E mass spectrometer.

**Benulin (I).** The stems, leaves, twigs, and bark of *Bursera arida*, collected in Tehuacan, Puebla, Mexico, during the month of June 1971, were ground in a Wiley Mill and stored at  $-10^\circ\text{C}$  prior to extraction.

The ground material (8 kg) was extracted exhaustively in a Lloyd-type extractor with chloroform. A 210-g portion of the air-dried chloroform extract was fractionated as outlined in Scheme I. Fraction



F, which on TLC showed the presence of benulin as one of the major spots, was then subjected to silica gel 60 column chromatography. Elution with 1% methanolic dichloromethane yielded fractions containing nearly pure benulin which were further purified by crystallization, giving benulin (I), mp  $281-283^\circ\text{C}$ , as cubelets from benzene-methanol. The IR [(KBr)  $3380$ ,  $1690$ ,  $1645$ , and  $880\text{ cm}^{-1}$ ], NMR [( $\text{CDCl}_3$ - $\text{CD}_3\text{COOD}$ )  $4.66-4.7$  (d, 2 H,  $\text{H}_2\text{C}=\text{CCH}_3$ ),  $1.68$  (s, 3 H,  $\text{H}_2\text{C}=\text{CCH}_3$ ),  $0.87$  (s, 3 H,  $\text{CH}_3$ ),  $0.97$  (s, 6 H, 2  $\text{CH}_3$ ), and  $1.02$  (s, 3 H,  $\text{CH}_3$ )], and mass [ $m/e$  470 ( $\text{M}^+$ ), 455, 452, 440, 313, 234, 205, 203, 189, and 187] spectra were in accord with structure I.

Anal. Calcd for  $C_{30}H_{46}O_4$ : C, 76.55; H, 9.85. Found: C, 76.42; H, 9.96.

Colorless crystals of benulin (I,  $C_{30}H_{46}O_4$ ) were grown from chloroform-benzene for an x-ray study. A prism of dimension  $0.3 \times 0.2 \times 0.4$  mm was mounted with the  $c$  axis parallel to the goniostat  $\phi$  axis. The space group was determined by film methods to be  $P2_12_12_1$ . The cell parameters were found by least-squares fitting of the settings for the four angles of seven reflections on a Picker-FACS-I diffractometer (Cu  $K\alpha$ ,  $\lambda = 1.54178$  Å, graphite monochromator) to be  $a = 19.2710$  (8),  $b = 19.1091$  (8), and  $c = 7.1356$  (3) Å. The crystal density was measured by flotation as 1.179 g/mL, agreeing well with a calculated density of 1.188 g/mL assuming four molecules in the unit cell. Intensity data were collected using a scintillation counter with pulse-height analyzer,  $\theta$ - $2\theta$  scan technique, maximum  $\sin \theta/\lambda$  0.562,  $2^\circ$ /min scan rate, 10-s background counts, attenuators when the count rate exceeded  $10^4$  counts/s, and  $2^\circ$  scan range with a dispersion factor allowing for  $\alpha_1$ - $\alpha_2$  splitting at large  $2\theta$  values. Of 2314 independent reflections measured,  $1979 > 3\alpha$  ( $I$ ) were considered observed. Three standard reflections were monitored every 50 measurements to check the crystal alignment and the stability; no decrease in the intensity of standards was observed. Lorentz and polarization corrections were applied to the data, but no correction was made for absorption.

Phases for reflections with normalized structure factor  $E > 1.4$  were generated using the direct method program MULTAN.<sup>5</sup> All nonhydrogen atoms were located on an  $E$  map using calculated phases as coefficients. Full matrix least-squares refinement in which positional and isotropic thermal parameters were varied reduced  $R$  to 0.117. Two more cycles of least-squares refinement using anisotropic thermal parameters reduced  $R$  to 0.096. A difference map at this stage revealed all the hydrogen atoms. One more cycle of least-squares refinement using anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors (of nonhydrogen atoms to which they were attached) for hydrogen atoms brought  $R$  to 0.061. Refinement was terminated at this stage since the ratios of shifts in parameters to estimated standard deviations were all less than 0.3. The refinement was based on  $F_o$ , the quantity minimized being  $\sum w(F_o - F_c)^2$ . The weighting scheme used was based on counter statistics as defined by Corfield et al.,<sup>6</sup> the value of  $p$  being 0.04. The scattering factors used were those of Hanson et al.<sup>7</sup> No correction was applied for extinction.

**Methyl Benulinate (Ia).** Esterification of I with diazomethane in the usual manner afforded Ia, mp 210.5–211.5 °C. The IR [(KBr) 1730 and 1210  $\text{cm}^{-1}$ ], NMR [3.63 (s, 3 H,  $\text{COOCH}_3$ )], and mass [ $m/e$  484 ( $M^+$ )] spectra were in accord with structure Ia.

**Dihydrobenulin (Ib).** Catalytic hydrogenation of benulin with platinum oxide in methanol gave dihydrobenulin, showing a negative tetranitromethane test. The IR [(KBr) 1380, 1360, and no absorption at 1645 and 880  $\text{cm}^{-1}$ ] and mass [ $m/e$  472 ( $M^+$ )] spectra were in accord with structure Ib.

**3-Keto-10-acetoxymethylbenulin (IIa).** Acetylation of benulin with acetic anhydride-pyridine on a steam bath afforded IIa, mp 120 °C (MeOH). The IR [( $\text{CCl}_4$ ) 1740, 1710, and 1690  $\text{cm}^{-1}$ ], NMR [1.93 (s, 3 H,  $\text{CH}_2\text{OCOCH}_3$ ), and mass [ $m/e$  512 ( $M^+$ ), 452, and no peak at 313] spectra were in accord with structure IIa.

The deuterated product of IIa, prepared according to the procedure

of Djerassi et al.,<sup>8</sup> displayed a molecular ion peak at  $m/e$  514 in the mass spectrum.

**10-Hydroxymethyl-3-deoxybenulin (IIb).** Huang-Minlon reduction of IIa with hydrazine hydrate and potassium hydroxide in ethylene glycol afforded IIb, mp 151–153 °C. The IR [(KBr) no absorption at 1740 and 1710  $\text{cm}^{-1}$ ] and mass [ $m/e$  456 ( $M^+$ ) and 425 (base)] spectra were in accord with structure IIb.

**Benulintriol (IIc).** Reduction of Ia with  $\text{LiAlH}_4$  in the usual way yielded a triol, mp 160–162 °C. The IR [(KBr) 3700–3100  $\text{cm}^{-1}$  and no carbonyl absorption], NMR [0.83 (s, 3 H,  $\text{CH}_3$ ), 1.08 (s, 6 H, 2  $\text{CH}_3$ ), 1.23 (s, 3 H,  $\text{CH}_3$ ), and 1.73 (s, 3 H,  $\text{CH}_3$ )], and mass [ $m/e$  458 ( $M^+$ ), 428, 427, 410, 409, 189, and 187] spectra were in accord with structure IIc.

**Benulintriol Triacetate (IId).** The triol IIc, on acetylation under conditions as described for IIa, yielded IId, mp 160–162 °C. The IR [(KBr) 1730, 1225  $\text{cm}^{-1}$  and no hydroxyl absorption], NMR [two acetyl methyl peaks (1 Hz apart) integrating for 9 protons], and mass [ $m/e$  524, 424, and 452; molecular ion peak at  $m/e$  584 was not observed] spectra were in accord with structure IId.

Anal. Calcd for  $C_{36}H_{56}O_6$ : C, 73.93; H, 9.65. Found: C, 74.19; H, 9.17.

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**Registry No.**—I, 59157-84-5; Ia, 59157-88-9; Ib, 61426-07-1; IIa, 61426-08-2; IIb, 61426-09-3; IIc, 61426-10-6; IId, 61426-11-7.

**Supplementary Material Available.** Tables of atomic coordinates, temperature factors, bond distances, bond angles, and torsion angles (6 pages). Ordering information is given on any current masthead page.

## References and Notes

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