Novel Ring Openings of Levopimaric Acid Salts[1a]

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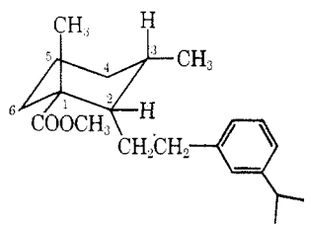
The reaction of levopimaric acid in the presence of 105 mol % potassium hydroxide gives four new compounds, three of which have been isolated and structures postulated.[2] The fourth compound has now been isolated and its structure postulated as indicated in **1**. In the previous work,[2] structure **2** was proposed



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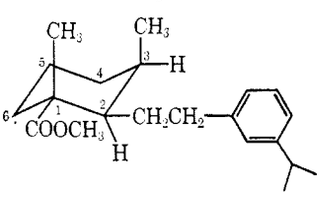

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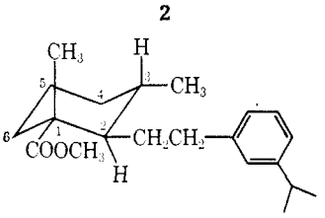

3

for the compound in which only ring B had been opened. Based on a comparison of the nmr spectrum of the two diastereomers, it is now postulated that the structure of the compound ("2") from the previous work[2] is as shown in **3**.

The infrared, ultraviolet absorption, and mass spectra of **1** are very similar to those of **3**. The detailed structure of **1** has been assigned on the basis of its nmr spectrum. The triplet with J = 9 Hz and centered at δ 2.61 was collapsed to a singlet by irradiating at a frequency corresponding to δ 1.41. This same irradiation frequency collapsed the structure at δ 2.05. Integration of the spectrum showed the triplet peaks at δ 2.61 to have a combined area equal to two protons and the absorption at δ 2.05 to have an area equal to one proton. The triplet at δ 2.61 was assigned to the two equivalent benzyl protons. Absorption at δ 1.41 was assigned to the methylene group located next to the benzyl group and the absorption at δ 2.05 was assigned to the tertiary proton at C-2. Following this, the doublet located with its center at δ 0.905 was decoupled by irradiating at a frequency corresponding to δ 1.53. The integration shows the doublet peaks to have a combined area equivalent to three protons. This doublet was thus assigned to the methyl group at C-3 and the unresolved multiplet at δ 1.53 was assigned to the tertiary proton at C-3. The doublet could be partially spin decoupled over a band of frequencies approximately 35 Hz wide. This is to be expected of the splitting of the C-3 proton by six other protons. The singlet absorption at δ 1.15 was assigned to the methyl group at C-1 and the singlet absorption at δ 3.66 was assigned to the methyl group of the ester.

A detailed consideration of compound **3** followed the same pattern as above. The triplet centered at δ 2.55 was assigned to the benzyl protons. This triplet could be collapsed to a singlet by irradiating at a frequency corresponding to δ 1.42. The absorption at δ 1.42 was assigned to the methylene group adjacent to the benzyl group. The absorption by the C-2 proton was not apparent by inspection and could not be located in the spin decoupling experiments. The doublet centered at δ 1.01 could be collapsed by irradiating at a frequency corresponding to 1.42 thus placing the C-3 proton at δ 1.42 with the accompanying assignment of the absorption at δ 1.01 to the C-3 methyl group.

The particular configurations were assigned for the following reasons. The tertiary proton on C-2 in compound **3** was placed at higher fields than the same proton in compound **1** because of the absence of any peaks in the range δ 1.8–2.4 and the absence of any unidentified absorption at still lower fields in **3**. This leads to the belief that the C-2 proton is axial in compound **3** and equatorial[3a] in compound **1**. It is probable that this proton cannot be found in the spectrum of **3** because of intensive splitting introduced by the axial C-3 proton.

The absorption of this proton on C-2 in compound **1** is poorly resolved but a definite triplet is apparent. This triplet has a J value of approximately 4 Hz and this splitting must be caused by interactions from the adjacent methylene group as shown during the spin decoupling. There is further splitting within the triplet which is poorly resolved even by spin decoupling. This has a J value of 2 Hz or less. The predicted value for J_{ea}^{vic} is 1.7 Hz.[4] Thus the C-3 proton is axial and the C-3 methyl group is equatorial.

The configuration at C-1 is the same in both compounds because the bands of these methyl groups do not change. The line width at half-height (W_H) of the resonance of the C-1 methyl group is 1.25 + 0.1 Hz for both **3** and **1**. The W_H for TMS was 0.5. According to Shoppee, *et al.*,[5] this is consistent with the axial nature of the C-1 methyl in both compounds. These methyl groups have the same chemical shift in

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(2) H. Takeda, W. H. Schuller, and R. V. Lawrence, *J. Org. Chem.*, **33**, 3718 (1968).

(3) (a) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. II, Pergamon Press, New York, N. Y., 1966, p 702; (b) p 701.

(4) J. I. Musher, *J. Chem. Phys.*, **34**, 594 (1961).

(5) C. W. Shoppee, F. P. Johnson, R. E. Lock, J. S. Shannon, and S. Sternhell, *Tetrahedron Suppl.*, **8**, 421 (1966); C. W. Shoppee, F. P. Johnson, R. L. Lock, and S. Sternhell, *Chem. Commun.*, **15**, 347 (1965).

both compounds, *i.e.*, 1.15 *vs.* 1.14 ppm. The constant W_H and δ would lead to the conclusion that there has been no change in the shielding of the C-1 methyl group, implying that the C-3 methyl group is equatorial in both cases. One would expect that an axial C-3 methyl would have strong interactions with the axial C-1 methyl and thus shift both the C-1 methyl and the C-3 methyl bands. The small differences in the δ values of the C-3 substituents between **1** and **3** (compound **1**, δ_H 1.53 and δ_{CH_3} 0.905; compound **3**, δ_H 1.42 and δ_{CH_3} 1.01) are attributed to differing effects from the β-phenethyl group.

Although it is tempting to assume that the downfield shift of the resonance of the C-3 methyl, which occurs between **1** and **3**, is indicative of a change from equatorial to axial conformation of this group, it is not possible to defend an inversion at C-3 using only this information. We, therefore, believe that the conformation at C-3 is the same in **1** and **3** (CH axial, CCH_3 equatorial).

The inversion of the hydrogen at C-3 in **3** may occur during the reaction of the C-3 anion with a proton.[2] The inversion of the hydrogen at C-2 in **1** may result from carbon–carbon cleavage between C-1 and C-2 of the resin acid molecule and a recombination during the reaction.[2] If this is the case, two more isomers could be formed. These isomers do not show up in the glpc analysis of the reaction mixture.

A comparison of the spectral data for **3** and the seco-dehydroabietate compound of Zinkel and Rowe[6] indicated that these compounds were apparently identical. Upon our isolation of **1**, however, a further more detailed comparison of the nmr spectra of **1** and the compound of Zinkel and Rowe showed the latter compounds to be identical. Zinkel, *et al.*, have confirmed this identity by glpc on DEGS and SE-30 columns[7] and concur with the assignment of structure **1**.

It was also found in the present work that methyl levopimarate refluxed in tri-*n*-butylamine for 5 hr gave the same four ring-opened compounds in 32.4% yield. No increase in yield was obtained on further refluxing.

Experimental Section[8]

Methyl 2α-[2′(*m*-Isopropylphenyl)ethyl]-1β,3α-dimethylcyclohexane Carboxylate (1).—Compound **1** was prepared by heating levopimaric acid with 105 mol % of potassium hydroxide for 3 days at 200° as described previously.[2] The compound of relative retention time 0.518 (methyl dehydroabietate, 1.0) was collected from a 10% Versamid 900 on Chromosorb W column (10 ft × 0.25 in. o.d. aluminum tubing F & M 500 instrument) run at 250°. Final purification was accomplished by very careful recollection from a 3.8% SE-30 Chromosorb W column (15 ft. × 0.25 in. o.d. aluminum column) at 200°. Analytical work was carried out on a 5% Versamid 900 on Chromosorb W (60–80 mesh) column (15 ft × 0.25 in. o.d. aluminum column) at 250°. Compound **1** was collected as a colorless oil which gave a single peak on both a Versamid 900 column (250°) and a 3.8% SE-30 column (at 200°). A 1:1 mixture of **2** and **1** was prepared and found to give two peaks on both a Versamid 900 and an SE-30 column. Compound **1** exhibits $[\alpha]^{25}D$ −13° (*c* 0.9, 95% EtOH); uv max (95% EtOH) 264 mμ (ε 284), 271 (253); ir (neat) 1725 (C=O), 1604 (aromatic), 1460 (CH_3), 1213, 1182, 1133 (isopropyl) 1112, 1048, 788 (*meta*-disubstituted aromatic); nmr ($CDCl_3$) δ 0.905 (d, 3, J = 7 Hz, C_3 CH_3), 1.15 (s, 3, C_1 CH_3), 1.245 (d, 6, J = 7 Hz, isopropyl CH_3), 1.41 (m, 2, C_2H CH_2), 1.53 (m, 1, C_3 **H**), 2.05 (t, 1, J = 4 Hz, C_2 **H**), 2.61 (t, 2, J = 9 Hz, C_6H_5 CH_2), 2.87 (quintet, 1, J = 7 Hz, *t*-**H** at isopropyl), 3.66 (s, 3, OCH_3), 7.02 largest peak (m, 4, aromatic **H**); mass spectrum (70 eV) *m/e* (relative intensity) 316 (15), 284 (15), 257 (3.9), 256 (3.2), 192 (7.9), 188 (6.3), 187 (39), 183 (7.9), 173 (3.2), 159 (3.2), 151 (7.9), 147 (15.8), 146 (100), 145 (3.9), 135 (3.9), 134 (28.3), 133 (45.6), 132 (3.9), 131 (14.2), 129 (3.2), 123 (18.9), 121 (3.2), 119 (7.1), 117 (18.9), 116 (7.1), 115 (5.5), 111 (7.9), 110 (3.2), 109 (12.6), 105 (11.8), 102 (3.2), 101 (45.6), 100 (3.2), 97 (3.2), 95 (11.8), 93 (6.3), 92 (23.6), 91 (25), 88 (3.2), 81 (11), 79 (5.9), 77 (3.9), 69 (8.7), 67 (7.9), 59 (3.9), 55 (14.2), 53 (3.2).

Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.72; H, 10.18. Found: C, 79.62; H, 10.23.

The nmr spectrum of **3** at 100 MHz showed δ 1.013 (d, 3, J = 5.5 Hz, C_3 CH_3), 1.14 (s, 3, C_1 CH_3), 1.235 (d, 6, J = 7 Hz, isopropyl CH_3), 1.42 (m, 2, C_2 CH_2), 1.42 (m, 1, C_3 **H**), <1.8 (definite position could not be found, C_2 **H**), 2.55 (t, 2, J = 7 Hz, $C_6H_5CH_2$), 2.86 (quintet, 1, J = 7 Hz, *t*-**H** at isopropyl), 3.64 (s, 3, OCH_3), 6.96 (m, 4, aromatic **H**).

Methyl Levopimarate Refluxed with Tri-*n*-butylamine.—Methyl levopimarate (0.8 g, 2.5 mmol) was dissolved with tri-*n*-butylamine (10 ml, 42 mmol) and refluxed (216°). After refluxing 5 hr, ether was added, the solution washed twice with aqueous acetic acid and with water five times, dried, and concentrated. The residue was analyzed by glpc (Versamid 900): methyl 9-(*m*-isopropylphenyl)-2,6-dimethyl-*cis*-6-nonenoate[2] (5.8%); mixture of **1** and methyl 9-(*m*-isopropylphenyl)-2,6-dimethyl-*trans*-6-nonenoate[2] (19.1%), **3** (1.75%), levopimarate–palustrate peak (39.8%), unknown (6.1%), dehydroabietate (19.5%), abietate (2.2%). The same composition was obtained on refluxing the sample for 10 hr.

Registry No.—**1**, 19556-80-0; **3**, 19556-81-1.

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(6) See ref 2, footnote 9.

(7) In the course of communications with D. F. Zinkel, an interpretation of our data was called to our attention which resulted in the postulation of **3** for the compound from ref 2.

(8) The nmr spectra were obtained using a Varian HA-100 nmr spectrometer. Chemical shifts were measured in deuteriochloroform as a solvent relative to tetramethylsilane (TMS) as an internal standard, δ_{TMS} = 0 ppm.

Tumor Inhibitors. XXXVI.[1] Eupatin and Eupatoretin, Two Cytotoxic Flavonols from *Eupatorium semiserratum*[2]

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During a recent study of the cytotoxic constituents of *Eupatorium* species,[3] the isolation and characterization of two previously unreported flavonoids were described. Flavanoid K, mp 243–245°, and flavonoid L, mp 146–148°, which we designate, respectively, as eupatin and eupatoretin, were found to show moderate cytotoxicity against human carcinoma of the nasopharynx carried in cell culture (KB).[3] We report here structural studies leading to assignment of the 3,5,3′-trihydroxy-6,7,4′-trimethoxyflavone structure (**1**) for

(1) Part XXXV in the series: S. M. Kupchan, P. S. Steyn, M. D. Grove, S. M. Horsfield, and S. W. Meitner, *J. Med. Chem.*, **12**, 167 (1969).

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(3) S. M. Kupchan, C. W. Sigel, R. J. Hemingway, J. R. Knox, and M. S. Udayamurthy, *Tetrahedron*, in press.