

# Bicoumol, a New Bicoumarinyl, from Ladino Clover

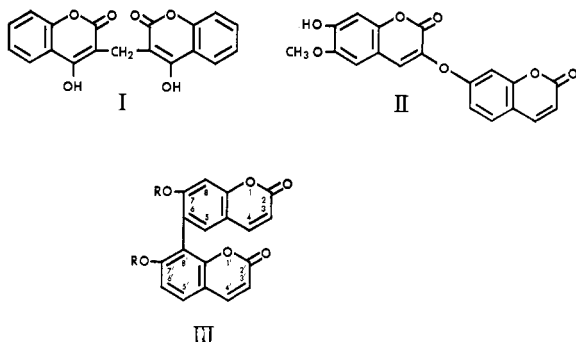
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The characterization of a novel coumarin, bicoumol, isolated from ladino clover as 7,7'-dihydroxy-6,8'-bicoumarinyl, is described. The new coumarin,  $C_{18}H_{10}O_6$ , was characterized by

proton magnetic resonance, ultraviolet, and infrared spectral studies. The presence of umbelliferone (7-hydroxycoumarin) in ladino clover is also reported.

The presence of coumarin-type compounds in plants has been well established. Coumarin dimers were first shown to occur naturally when Link (12) reported the structure of 3,3'-methylenebis(4-hydroxycoumarin) [dicoumarol (I)] as two coumarins connected by a methylene group. A different type of coumarin dimer was reported by Tschesche and co-workers (13). They identified daphnoretin (II) (8) as two coumarins linked by an ether bridge. Mihashi and co-workers (9, 10) have recently isolated a third type of coumarin dimer in which the two coumarins are linked through a carbon-carbon bond. They named their compound Matsukaze-lactone (III,  $R = CH_3$ ).

Synthetic compounds of the latter structure are known and are called bicoumarinyls. They include 3,4'-bicoumarinyl (2), 4,4'-dihydroxy-3,3'-bicoumarinyl (4), 7,7'-dimethoxy- or dihydroxy-8,8'-bicoumarinyl (7) and 6,6'-dimethoxy-5,5'-bicoumarinyl (11).



The simple coumarin, umbelliferone, which occurs widely in nature, has not been reported as a constituent of ladino clover. During the isolation of phenolic compounds from ladino clover, two compounds designated as compound V and VIII were obtained (1). This paper reports the structure of compound VIII as umbelliferone and compound V as 7,7'-dihydroxy-6,8'-bicoumarinyl (III,  $R = H$ ), for which the name bicoumol is proposed.

## Experimental

**Bicoumol.** ISOLATION. The isolation of bicoumol (compound V), 470 mg., by countercurrent distribution (CCD) from an acetone extract of ladino clover has been described (1). Recrystallization of bicoumol from ethanol and acetone gave an analytical sample, 52 mg., m.p. 293–94° C.  $\lambda_{\text{max}}^{\text{EtOH}}$  258 m $\mu$  (shoulder), 328 m $\mu$ .  $\lambda_{\text{max}}^{\text{EtOH-NaOAc}}$  350 m $\mu$ . The infrared spectrum showed bands at 3370  $\text{cm}^{-1}$  (hydroxyl), 3270  $\text{cm}^{-1}$  (CH), and 1709  $\text{cm}^{-1}$  (lactone carbonyl).

Calculated for  $C_{18}H_{10}O_6$ : C, 67.08; H, 3.13. Found: C, 66.7; H, 3.18.

**Diacetate.** Bicoumol, 100 mg., was refluxed with anhydrous sodium acetate, 250 mg., and acetic anhydride, 5.0 ml., for 5 minutes, cooled, and poured into cold water giving 90 mg. of a white solid. An analytical sample (m.p. 228.5–229.5° C.) was prepared by recrystallization from acetone–water.  $\lambda_{\text{max}}^{\text{EtOH}}$  281, 314 m $\mu$ . The infrared spectrum showed bands at 1770  $\text{cm}^{-1}$  (acetyl carbonyl), 1741  $\text{cm}^{-1}$  (lactone), and 1198  $\text{cm}^{-1}$  (acetyl carbonyl).

Calculated for  $C_{22}H_{14}O_8$ : C, 65.03; H, 3.47;  $CH_3CO$ , 21.2. Found: C, 65.2; H, 3.54;  $CH_3CO$ , 21.3.

**Dimethyl Ether.** Bicoumol, 100 mg., anhydrous potassium bicarbonate, 200 mg., and dimethylsulfate, 0.25 ml., were refluxed in dry acetone, 25.0 ml., for 3 hours, cooled, and poured into water. The white solids were collected and twice recrystallized from methanol to give colorless platelets, 51 mg., m.p. 266.5–267.5° C.  $\lambda_{\text{max}}^{\text{EtOH}}$  252, 327 m $\mu$ . The infrared spectrum showed bands at 1727  $\text{cm}^{-1}$  (lactone carbonyl) and 1206, 1195  $\text{cm}^{-1}$  (methoxyl).

Calculated for  $C_{20}H_{14}O_6$ : C, 68.5; H, 4.03;  $OCH_3$ , 17.7. Found: C, 68.1; H, 4.07;  $OCH_3$ , 18.0.

**7-Hydroxycoumarin.** ISOLATION. The isolation of (compound VIII), 800 mg., by CCD from an acetone extract of ladino clover has been described (1). Recrystallization of a portion from methanol gave an analytical sample, 40 mg., m.p. 232–33.5° C.; lit. m.p. 232° C. (3).

Calculated for  $C_9H_6O_3$ : C, 66.7; H, 3.73. Found: C, 66.9; H, 3.79.

**ACETATE.** Compound VIII, 125 mg., was refluxed with fused sodium acetate, 300 mg., and acetic anhydride, 6.0 ml., for 5 minutes, cooled, and poured into cold water, giving 130 mg. of a white solid. Recrystallization from boiling water gave 95 mg. of a white solid,

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m.p. 140.5-141° C.; lit. m.p. 140° C. (3).

Calculated for  $C_{11}H_8O_4$ : C, 64.7; H, 3.95;  $CH_3CO$ , 20.6. Found: C, 64.8; H, 4.01;  $CH_3CO$ , 21.1.

### Results and Discussion

The similarity of the ultraviolet spectrum of bicoumol with that of umbelliferone (7-hydroxycoumarin) first suggested that it was a closely related compound. However, the 60-MHz. proton magnetic resonance (PMR) spectrum of bicoumol in dimethylsulfoxide, while similar to that of umbelliferone, integrated for eight aromatic protons instead of the five protons expected for a simple monohydroxy coumarin. The infrared spectrum suggested a coumarin dimer by the presence of a relatively wide carbonyl band at  $1709\text{ cm}^{-1}$ , since daphnoretin (II), which contains two lactone groups, also has a broad carbonyl band in this area. A coumarin dimer structure with a carbon-carbon linkage was further suggested by mass spectrometry which gave a molecular ion peak at  $m/e$  322. Applying a carbon-carbon linked coumarin dimer to the elemental analysis of bicoumol and its derivatives indicated that it contained two hydroxyl groups and had an empirical formula of  $C_{18}H_{10}O_8$ . This gives a molecular weight of 322, which agrees with the mass spectra data.

The  $\lambda_{\text{max}}$  of bicoumol in ethanol ( $328\text{ m}\mu$ ) underwent a bathochromic shift to  $350\text{ m}\mu$  in the presence of sodium acetate, an indication that at least one of the hydroxyl groups is in the 7-position on one of the coumarin rings (6). No further bathochromic shift was obtained with boric acid-sodium acetate. Therefore, the two hydroxyl groups are not ortho to each other (5).

The eight protons of bicoumol were resolved with a 100-MHz. PMR spectrum (Figure 1) of bicoumol in dimethylsulfoxide. Two hydroxyl protons were seen downfield in the PMR spectrum, but were not reproduced in Figure 1. The spectrum shows six ortho-split doublets (6 protons) and two singlets (2 protons). The two high-field ortho doublets ( $\tau = 3.79$  and  $3.82$ ;  $J = 9.5\text{ Hz.}$ ) can be assigned to the 3- and 3'-positions in the B-rings of the dimer. The remaining protons in the B-rings (4- and 4'-position) can be assigned to the two low-field doublets ( $\tau = 2.02$  and  $2.03$ ). Therefore, the two hydroxyl groups (established chemically) must be in the A-rings in addition to the carbon-carbon bridge. The spin-spin splittings show that three of the remaining four protons cannot be in one of the A-rings. Therefore, the third ortho doublet must arise from a proton in one A-ring and the two singlets from protons in the other. The two singlet peaks ( $\tau = 2.52$  and  $3.12$ ) are not meta coupled and must, therefore, be located para to each other. Most naturally occurring coumarins contain oxygen functional groups at the 7-positions. By making this assumption and assigning the hydroxyls to the 7- and 7'-position, the remaining ortho doublets ( $\tau = 2.46$  and  $3.06$ ;  $J = 8.5\text{ Hz.}$ ) can be assigned to the 5- and 6-positions of one A-ring and the singlets to the 5- and 8-positions in the other. The rings would then be bonded between the 6- and 8'-positions. The proton assignments are shown in Figure 1.

Thus, the structure most compatible with the PMR

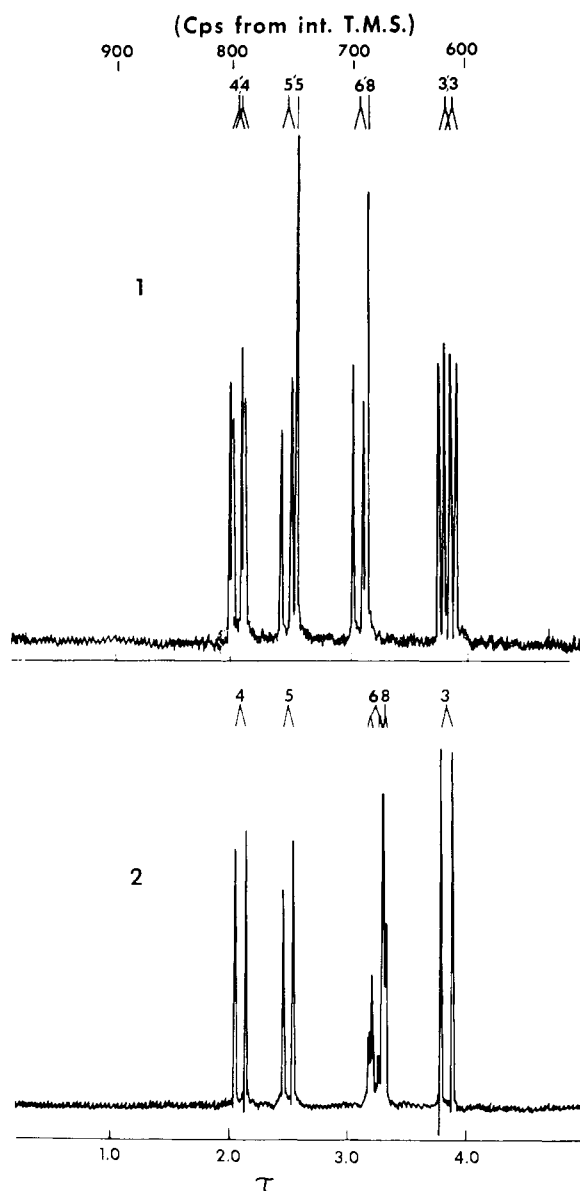


Figure 1. Proton magnetic resonance spectrum (100 MHz.) of (1) bicoumol and (2) umbelliferone, both in  $d_6$ -dimethylsulfoxide

data is 7,7'-dihydroxy-6,8'-bicoumarinyl. This structure would make Matsukaze-lactone (III,  $R = CH_3$ ) the dimethyl ether of bicoumol. This was confirmed by the comparison of the PMR, infrared, and ultraviolet spectra of bicoumol dimethyl ether with authentic Matsukaze-lactone. They were identical in all respects.

Comparison of the PMR, infrared, and ultraviolet spectra of compound VIII and its acetate with authentic umbelliferone and its acetate proved them to be identical.

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