

EUPARINOID CONSTITUENTS OF *ENCELIA CALIFORNICA*

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Abstract—*Encelia californica* Nutt. (Compositae, tribe Heliantheae) contains euparin and two new compounds of related constitution. No sesquiterpene lactones were found.

INTRODUCTION

IN AN earlier phase of a study of plants of the tribe Heliantheae, *Encelia farinosa* Gray was found to contain sesquiterpene lactones of the class common to many genera of Compositae.^{1,2} Examination of the related species, *E. californica* Nutt. has now disclosed that this plant contains benzofuran and chromene derivatives. No sesquiterpene lactones were found.

RESULTS AND DISCUSSION

E. californica, an abundant perennial of the coastal region of Southern California, contains the known euparin (I),³⁻⁷ along with two new compounds that bear an obvious biogenetic relationship to euparin.

Encocalin (II), the major constituent of the plant, is a yellow oil which formed a crystalline oxime, analysis of which indicated that encocalin has the composition C₁₄H₁₆O₃, a result confirmed by the appearance of the molecular ion at *m/e* 232 in the mass spectrum. The i.r. spectrum of encocalin showed peaks at 1675 and between 1500 and 1625 cm⁻¹, indicative of an aromatic ketone, and the NMR spectrum was clearly interpretable in accord with the structure II. Sharp singlets at δ 1.41 (6 H), 2.52 (3 H) and 3.82 (3 H) corresponded to the 2,2-dimethyl grouping of the chromene ring, the acetyl group and the methoxyl group. The aromatic hydrogen atoms appeared as one-proton singlets at δ 6.38 (H-7) and 7.50 (H-5), showing that the unoccupied positions on the ring were in *para* positions. The hydrogen atoms at C-3 and C-4 appeared as a symmetrical AB pattern of doublets at δ 5.48 and 6.28 (one proton each, *J* = 10 Hz). These data led to the formulation of encocalin as II, a conclusion that was substantiated by the conversion of dihydroencocalin into the known acid, III, by means of hypochlorite oxidation.⁸

Euparin (I) was identified by its composition, m.p., characteristic green ferric reaction

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¹ T. A. GEISSMAN and R. MUKHERJEE, *J. Org. Chem.* **33**, 656 (1968).

² W. HERZ, P. S. SUBRAMANIAN and T. A. GEISSMAN, *J. Org. Chem.* **33**, 3743 (1968).

³ B. KARNTHONG and A. ROBERTSON, *J. Chem. Soc.* **925** (1939).

⁴ F. GIZYCKI, *Suddentsch. Apoth. Z.* **90**, 503 (1950).

⁵ Z. JERMANOWSKA, *Polsk. Akad. Umiejtnosci; Prace Komisji Nauk. Farm., Diss. Farm.* **3**, 165 (1951); *Chem. Abs.* **48**, 5848 (1954).

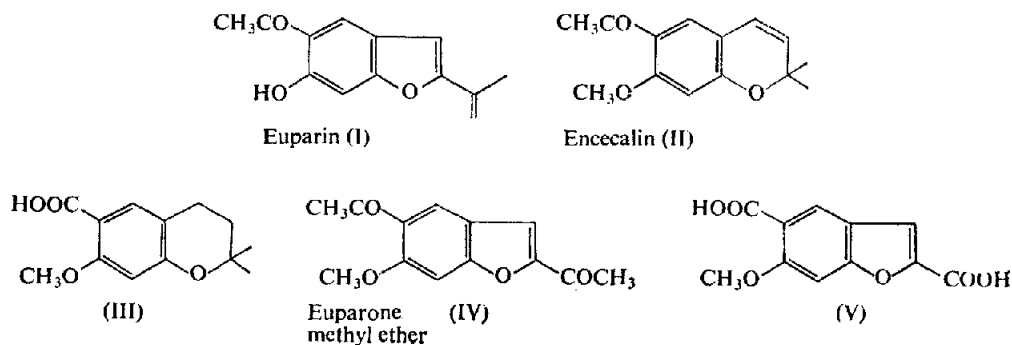
⁶ J. SYKULSKI, *Acta Polon. Pharm.* **15**, 361 (1958); *Chem. Abs.* **53**, 6536 (1959).

⁷ T. NAKAOKI, N. MARITA and S. NISHINO, *Yakugaku Zasshi* **78**, 557 (1958).

⁸ J. CHATTERJEE, K. BANERJI and N. PRASAD, *Chem. Ber.* **96**, 2356 (1963).

and the m.p. of its monoacetate.³ Its NMR spectrum showed a one-proton singlet at δ 12.4 (removed by deuterium oxide), characteristic of the hydroxyl group of an *o*-hydroxy ketone; three one-proton singlets for the protons at C-4, -3 and -7 at δ 7.88, 6.88 and 6.45; two broadened one-proton singlets at δ 5.15 and 5.70 (methylene group), and three-proton signals for the vinylic methyl group (δ 2.05, doublet, $J=1$ Hz) and for the acetyl group (δ 2.60, singlet).

Euparone methyl ether (IV), $C_{13}H_{14}O_2$, is a pale yellow, crystalline compound, m.p. 140–142°. Its mass spectrum showed the molecular ion at m/e 232 and a prominent peak at m/e 43, characteristic of the acetyl group. Intense maxima were present in the u.v. spectrum at 231 ($\log \epsilon$ 4.11), 250 (4.23), 298 (4.11) and 330 nm (4.21), and the i.r. spectrum showed peaks at 1680 and between 1400 and 1625 cm^{-1} . The NMR spectrum was remarkably simple: it showed six singlets, two at δ 2.55, 2.61 (3 H each, two acetyl groups), one at 3.97 (3 H, methoxyl), and three of one proton each at δ 7.08 (H-7), 7.42 (H-4) and 8.03 (H-3). These data are accommodated in the structure IV, and this was shown to be correct by the oxidation of euparone methyl ether to the known dicarboxylic acid V.⁹ The acid (V) was synthesized by way of 6-methoxycarbonyl-7-methoxycoumarin, and was converted into euparone methyl ether, identical with the natural compounds, by means of methyllithium.



At the time its structure was established, euparone methyl ether was the only 2-acetyl-coumarone derivative of this class known in nature. The occurrence of the demethoxy compound, 2,5-diacetylcoumarone, from an *Aplopappus* species, has recently been reported.¹⁰

The biosynthetic relationships between the compounds present in *E. californica* are obvious from their structures and are in accord with accepted views concerning the common origin of the 2-isopropylcoumarone and 2,2-dimethylchromene structures; namely, that they derive from an isoprenoid progenitor that undergoes ring closure in two ways. The formation of the 2-acetylcoumarone from a 2-isopropenyl precursor seems clearly to be the consequence of the oxidative removal of the exocyclic methylene group.

The structural relationships between the 5-acetylbenzofuranoid constituents of *Encelia* (tribe Heliantheae), *Eupatorium* (tribe Eupatorieae) and *Aplopappus* (tribe Asterae),¹¹ and in

⁹ S. LELE, M. PATEL and S. SETHAN, *J. Indian Chem. Soc.* **37**, 775 (1960).

¹⁰ L. H. ZALKOW, G. A. CABOT, G. L. CHETTY, M. GHOSAL and G. KEEN, *Tetrahedron Letters* 5727 (1968).

¹¹ The latter two genera contain several members of the group of euparinoid compounds typified by tremetone and its allies; see W. BONNER, N. BURKE, W. FLECK, R. HILL, J. JOULE, B. SJOBERG and L. H. ZALKOW, *Tetrahedron* **20**, 1419 (1964).

particular the presence of the unique 2-acetyl derivatives in *Encelia* and *Aplopappus*, suggest an alliance between these tribes that may prove to be of significance in the assessment of the phylogenetic relationships in Compositae. Further study of additional *Encelia* species is in progress.

EXPERIMENTAL

M.ps were taken in capillary tubes and are corrected. NMR spectra were measured in CDCl_3 with a Varian A-60 instrument. Mass spectra were recorded with an AEI MS-9 spectrometer operating at 70 eV with the direct insertion technique. TLC was carried out with silica gel plates developed with CHCl_3 -acetone (4:1).

Extraction of Plant Material

Encelia californica Nutt. was collected in the lower Santa Monica mountains in May, 1968. Leaves were stripped from woody stems, dried, and ground to a powder. The extraction of 6100 g of plant with CHCl_3 at room temperature, followed by removal of solvent under reduced pressure, yielded a tarry residue which was extracted by agitation with 2000 ml of a mixture of ethanol-water (1:2). The clarified aqueous part was extracted with CHCl_3 and the dried extract (MgSO_4) evaporated to yield 63 g of a brown oil. The crude oily material was chromatographed over 1260 g of silica gel with CHCl_3 , with the collection of 250-ml fractions. Evaporation of fractions 4-8 yielded a crystalline residue, which upon recrystallization from benzene-pentane afforded 93 mg of euparin. Fractions 9-30 yielded 5 g of encocalin, and fractions 88-105 afforded 550 mg of euparone methyl ether. Since TLC showed that the tarry residue from the ethanol-water extraction still contained much encocalin, the tar was diluted with CHCl_3 , and, after drying, the solution was evaporated and the residual tar distilled under reduced pressure. An additional 48 g of encocalin was obtained, making the total of this compound 53 g, or 0.87 per cent of the dry plant.

Euparin (I)

Euparin was crystallized from benzene-pentane as yellow spears, m.p. 116-117° (lit.,³ m.p. 118-5°). It gave a green color with FeCl_3 . Anal. Calc. for $\text{C}_{13}\text{H}_{12}\text{O}_3$: C, 73.02; H, 6.13. Found: C, 72.83; H, 5.93 per cent. *Euparin acetate* was prepared by acetylation of euparin with acetic anhydride-pyridine in the usual way. The colorless material had m.p. 77-79° (lit.,^{3,7} m.p. 80°; 79°). The mass spectrum showed the molecular ion at the expected (for $\text{C}_{15}\text{H}_{14}\text{O}_4$) value of m/e 258. *Euparone methyl ether* (IV) crystallized from ether-pentane as pale yellow needles, m.p. 140-141°. Anal. Calc. for $\text{C}_{13}\text{H}_{12}\text{O}_4$: C, 67.23; H, 5.21. Found: C, 67.24; H, 5.39 per cent. The compound was neutral and gave positive tests for the presence of a carbonyl group. Its spectral properties are described above. *Euparone methyl ether bis-2,4-dinitrophenylhydrazone* was prepared in the usual way, and had m.p. 270-272° (from aqueous ethanol). Anal. Calc. for $\text{C}_{25}\text{H}_{20}\text{H}_{10}\text{O}_8$: C, 50.68; H, 3.40; N, 18.91. Found: C, 50.58; H, 3.60; N, 18.61 per cent.

6-Methoxycoumarone-2,5-Dicarboxylic Acid (V)

A. From euparone methyl ether (IV). A solution of 50 mg of IV in 10 ml of dioxane was added slowly with stirring to an ice-cold solution of NaClO . The resulting solution was stirred overnight and allowed to come to room temperature, the excess oxidant was destroyed with sodium bisulfite, and the solution acidified. The precipitate was collected and recrystallized from aqueous ethanol, from which it formed white needles, m.p. 286-287° (reported,¹² 285°). Mixed with the synthetic material (see part B). The m.p. was not depressed. Anal. Calc. for $\text{C}_{11}\text{H}_8\text{O}_6$: C, 55.94; H, 3.41; Neutr. Eq., 118. Found: C, 56.24; H, 3.52 per cent, N.E., 119.

B. By synthesis. 6-Methoxycarbonyl-7-methoxycoumarin was prepared by the condensation of malic acid and β -resorcylic acid as described by Kumar *et al.*¹² The product (m.p. 171-172°; reported¹² 172°) was converted into the dicarboxylic acid (V) in 69 per cent yield by treatment with Br_2 followed by NaOH .¹² The synthetic acid (V) had m.p. 286-287° and did not depress the m.p. of the acid prepared from the natural compound.

Euparone Methyl Ether (IV) from (V)

A solution of 1.5 g of the dicarboxylic acid (V) in anhydrous THF was added slowly to a stirred ethereal solution of LiMe . After 3 hr the reaction mixture was decomposed with aqu NH_4Cl . Evaporation of the ether layer yielded a solid which was recrystallized from ether-pentane to yield euparone methyl ether, m.p. and mixed m.p. with the natural compound, 140-141°. Anal. Calc. for $\text{C}_{13}\text{H}_{12}\text{O}_4$: C, 67.23; H, 5.21. Found: C, 67.21; H, 5.29 per cent.

¹² S. KUMAR, L. RAM and J. NATH RAY, *J. Indian Chem. Soc.* **23**, 365 (1946).

Encecalin (II)

The yellow, oily compound had b.p. 123° (0.05 mm) which, although homogeneous on TLC, did not give a satisfactory elemental analysis. Its mass spectrum showed the molecular ion at m/e 232, in agreement with the composition $C_{14}H_{16}O_3$. The NMR spectrum has been described in the Discussion. *Encecalin oxime*, prepared in the usual way, formed colorless needles, m.p. 140°. *Anal.* Calc. for $C_{14}H_{17}NO_3$: C, 67.99; H, 6.93. Found: C, 67.93; H, 6.61 per cent. The NMR spectrum of the oxime showed the features expected for the structure II for encecalin. The aromatic proton at C-5, found as a singlet at δ 7.50 in encecalin, is shifted to δ 6.94 in the oxime, an observation that serves to identify the signal for the proton in this position in the compounds of this series.

7-Methoxy-2,2-Dimethylchroman-6-Carboxylic Acid (III)

Hydrogenation of encecalin (1.0 g) in ethyl acetate in the presence of Pd-C resulted in the rapid uptake of 1 mole of H_2 . The dihydro compound was not characterized but, after removal of the solvent, was oxidized directly with NaClO. The recrystallized acid formed colorless prisms, m.p. 115–116° (reported,⁸ m.p. 116°). *Anal.* Calc. for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 65.95; H, 6.79 per cent. The NMR spectrum of the acid was in accord with the structure III. It showed the following signals: δ 7.60 (1 H, singlet, H-5); 6.34 (1 H, singlet, H-8); 3.80 (3 H, singlet, methoxy); 2.75 (2 H, triplet, $J=6$ Hz, H-4); 1.80 (2 H, triplet, $J=6$ Hz, H-3); and 1.35 (6 H, singlet, 2 CH_3 at C-2).

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