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EUCRYPHIN, A NEW CHROMONE RHAMNOSIDE FROM THE BARK OF *EUCRYPHIA CORDIFOLIA*

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Key Word Index—*Eucryphia cordifolia*; Eucryphiaceae; chromone rhamnoside; carbon-13 NMR.

Abstract—Eucryphin, a new chromone-3α-rhamnoside was isolated from the bark of *Eucryphia cordifolia* and its constitution and configuration clarified by ^{13}C NMR spectroscopy. Two other isolated products were identified as the known flavonoids astilbin and engelitin.

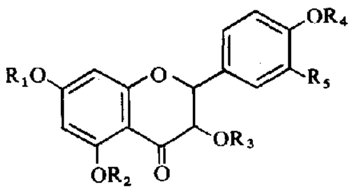
INTRODUCTION

Eucryphia cordifolia Cav. is a tree growing in the south of Chile and called ulmo. It belongs with four other species from Chile, Australia and Tasmania to the family Eucryphiaceae [1]. Following Bausch [2], Eucryphiaceae is closely related to Cunoniaceae. So far, only a few chemical investigations deal with both families [3].

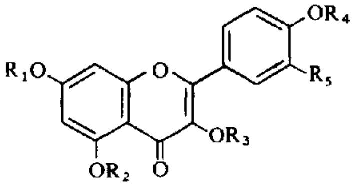
RESULTS AND DISCUSSION

The only study on Eucryphiaceae available yet [4] proved exclusively that the leaves of the species originating from Chile contain azaleatin (**4**) and caryatin (**5**). Thus, the authors concluded that the distinct chemical differences characterize the geographic origin of the plants.

However, no methoxy derivative of quercetin (**6**) could be identified in the bark of Eucryphiaceae. Astilbin (**2**) (3-*O*-α-L-rhamnoside of dihydroquercetin (**7**)) was identified as the main constituent. Additionally, engelitin (**3**) (3-*O*-α-L-rhamnoside of dihydrokaemperol (**8**)) and a glycoside (**1**), now called eucryphin, with molecular formula $C_{15}H_{16}O_9$, were isolated.



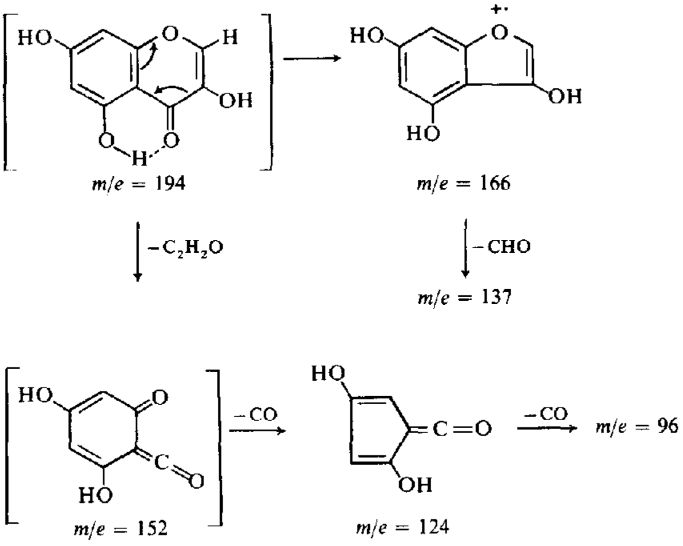
Astilbin 2 $R_1 = R_2 = R_4 = H; R_3 = Rha; R_5 = OH$
Engelitin 3 $R_1 = R_2 = R_4 = R_5 = H; R_3 = Rha$
Dihydroquercetin **7** $R_1 = R_2 = R_3 = R_4 = H; R_5 = OH$
Dihydrokaempferol **8** $R_1 = R_2 = R_3 = R_4 = R_5 = H$

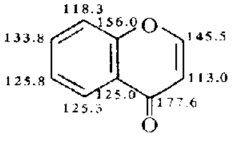


Azaleatin **4** $R_1 = R_3 = R_4 = H; R_2 = Me; R_5 = OH$
Caryatin **5** $R_1 = R_4 = H; R_2 = R_3 = Me; R_5 = OH$
Quercetin **6** $R_1 = R_2 = R_3 = R_4 = H; R_5 = OH$
Kaempferol **9** $R_1 = R_2 = R_3 = R_4 = R_5 = H$

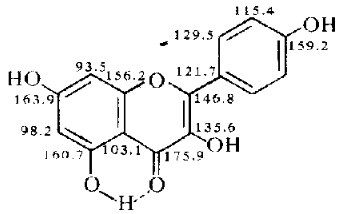
Strong IR absorptions of eucryphin at 3455, 3370 and 1645 cm^{-1} identified OH-groups and the carbonyl function of a chromone or flavonoid system [5]. Two other bands (1610 and 1580 cm^{-1}) appeared to be characteristic of an aromatic ring in conjugation to a carbonyl group. A benzo-γ-pyrone system is also obvious from the UV spectrum with maxima at 328 nm.

The following fragmentation pattern can be deduced from the mass spectrum [6]:

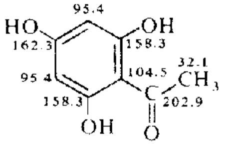




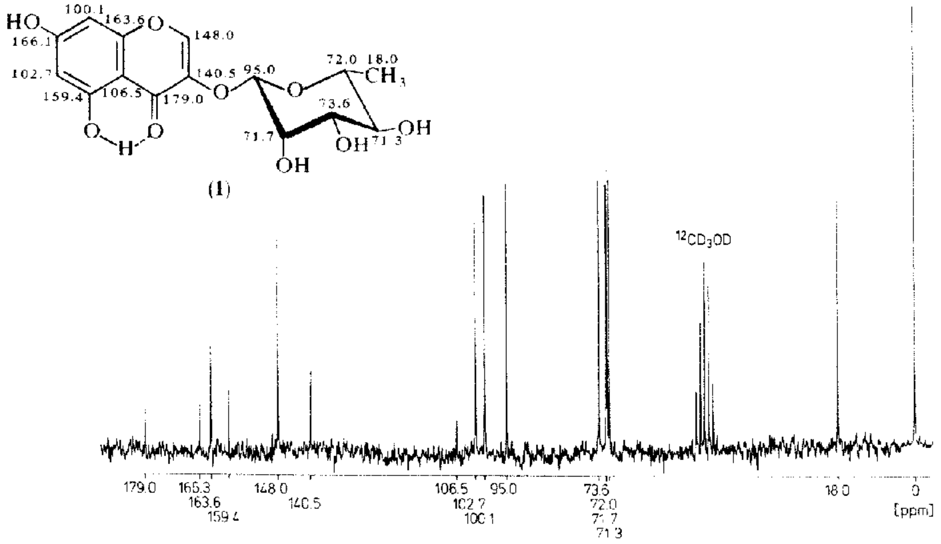
Chromone



Kaempferol



2,4,6-Trihydroxyacetophenone



(1)

Fig. 1. ^{13}C NMR spectrum (22.63 MHz) of eucryphin in tetradeuteriomethanol, 7 mg/1 ml, 120000 scans, proton broad-band decoupled. The assignments C5 ⇌ C9 may be interchanged [9].

The ^1H NMR spectrum in tetradeuteriomethanol showed an AB system for aromatic protons, formed by two doublets at δ 6.15 and 6.38 ppm with a *meta* coupling of J_m = 2.1 Hz. Another singlet for one proton located at δ 8.12 ppm was slightly deshielded relative to the value usually observed for H-2 of a chromone (δ 7.88 ppm) [7]. A doublet at δ 1.28 ppm with J = 6.0 Hz (3 protons) identified the methyl group of a 6-desoxy sugar residue which was found to be α-L-rhamnose by enzymatic cleavage.

The final structural assignment was achieved by ^{13}C NMR. The carbon shifts measured for eucryphin can be approached by applying the OH increments for benzene carbons [8] and using chromone as the parent compound. Further, the shift values of eucryphin were similar to those described for kaempferol (**9**) and 2,4,6-trihydroxyacetophenone [8–10]. A deshielding of C-3 (140.5 ppm in **1** relative to 135.6 ppm in **9**) arises from glycosidation. The signals at 95.0, 73.6, 72.0, 71.7, 71.3 and 18.0 ppm agree well with the data published for α-L-rhamnose [11]. The ^{13}C NMR spectrum with the complete assignments for all carbons is shown in Fig. 1. To conclude, eucryphin(**1**) is 5,7-dihydroxy-3-(α-*O*-L-rhamnopyranosyl)-4H-1-benzopyran-4-one.

EXPERIMENTAL

Mps were determined with the Kofler–Weygand microscope. Chromatographic separations were achieved with silica PF_{254} (TLC) and $PF_{254+366}$ (DC) (Merck), unsieved silica of Hermann, Cologne (SC), Sephadex LH-20 of Pharmacia, Uppsala, and paper 2043b Mgl of Schleicher & Schüll. Solvent systems were $CHCl_3$–MeOH–H_2O 1 (74:23:3), 2 (65:23:3) and 3 (65:30:10).

The MeOH extract (A) obtained from 1 kg bark of *Eucryphia cordifolia* was used as starting material. 100 g were dissolved in H_2O (2 l.), extracted twice with EtOAc (1 l.), and then exhaustively with *n*-BuOH. Both extracts exhibited an intense fluorescence on TLC so that they were treated together in the following (extract B).

35 g extract B were separated in a column filled with 1 kg silica by eluting with $CHCl_3$–MeOH–H_2O (30:3:1). After an elution of 10 l., the polarity was increased by 100 ml MeOH. 200 ml fractions were collected of which the 21st 24th were proved by TLC (systems 1–3) to contain 3 partly separated substances with R_fs from 0.4 to 0.6. Evapn of these 4 fractions to dryness yielded 8 g extract C.

5 g extract C were dissolved in 20 ml MeOH and separated on 200 g Sephadex. U-12 (*ca* 1.5 mg), U-10/U-9 (*ca* 200 mg) and U-12/U-10/U-9 (*ca* 300 mg) were obtained. The mixture U-10/U-9 was separated by PLC with solvent 1.

Eucryphin (**1**). U-9: mp 231–233°; $[\alpha]_D^{20}$ −187.5° (c = 0.466 in MeOH). UV (MeOH): λ_{max} (log ε): 252 (4.351), 260 (4.327, sh), 298 (3.898) and 328 nm (3.66). ^1H NMR (CD_3OD, 90 MHz, PFT): δ 1.25 (d, J_{vic} = 6 Hz, 3H, CH_3 of α-L-rhamnose), 6.15 (d, J_m = 2.1 Hz, 1 H at C-6), 6.38 (d, J_m = 2.1 Hz, 1 H at C-8), 8.12 ppm (s, 1 H at C-2). FI–MS (150°, DE, 70 eV): m/e 340 (M^+), 237, 223, 207, 194 (100%), 166, 152, 137, 124, 85, 69, 57, 43. ($C_{15}H_{16}O_4$ (1 × H_2O) calc: C, 50.2; H, 5.02. Found: C, 49.9; H, 5.04%).

The enzymatic cleavage of U-9 with the enzyme mixture EL

67-27 (Röhm & Haas, containing β-glycosidase, α-rhamnosidase and β-xylosidase) yielded 3,5,7-trihydroxy-4H-1-benzopyran-4-one, mp 244–246°. ^{1}H NMR ($(CD_3)_2CO$, 90 MHz, PFT): δ 6.26 (*d*, $J_m = 2$ Hz, 1 H at C-6), 6.39 (*d*, $J_m = 2$ Hz, 1 H at C-8), 7.76 (*br s*, 1 H, exchangeable with D_2O, C-7-OH), 8.02 (*s*, 1 H at C-2), 9.69 (*br s*, vanishes after shaking with D_2O, C-3-OH), 12.2 ppm (*s*, 1 H, vanishes after shaking with D_2O, C-5-OH). EI–MS (DE, 150°, 70 eV): *m/e* 195 ($M^+ + H$, 100%), 166 (M – CO, 10), 152 ($M - C_2H_2O$, 5), 137 (60), 124 (5).

Astilbin (**2**). *U*-12: mp 181–183° (from H_2O) (ref. 179–180°). UV (MeOH): λ_{max} (log ε): 235 (4.198), 292 (4.272) and 335 nm (3.427). IR (KBr): 3680–3100 (OH), 1640 (C=O), 1580, 1500 (phenyl), 1240, 1150, 1020 cm^{-1}. ^{1}H NMR (Me_2CO d_6, 90 MHz, PFT): δ 1.18 (*d*, $J_{vic} = 6$ Hz, 3H(CH_3)) of α-L-rhamnose, 4.72 (*d*, $J_{vic} = 10$ Hz, 1 H at C-3), 5.25 (*d*, $J_{vic} = 10$ Hz, 1 H at C-2), 6.00 (*d*, $J_m = 1.5$ Hz, 1 H at C-6), 6.05 (*d*, $J_m = 1.5$ Hz, 1 H at C-8), 6.92 (2 H at C-2′ and C-6′, AB-system, *ca* 2 Hz), 7.12 (1 H at C-5′), 11.97 (*s*, 1 H exchangeable with D_2O, C-5-OH).

U-12-acetate: mp 105–107°; $[\alpha]_D^{20}$ $-65.4°$ ($c = 1$ in chloroform). No OH absorptions were observed in the IR spectrum obtained from a KBr pellet. ^{1}H NMR ($CDCl_3$, 90 MHz, PFT): δ 7.43, 7.37, 7.31,6.82, 6.63, 5.43–4.90, 4.43, 3.87, 2.40, 2.32, 2.29, 2.05, 2.01, 1.96 and 1.12 ppm. These proton shifts agree with the values reported for astilbin peracetate [12]. Enzymatic and acidic cleavage of U-12 yielded taxifolin and α-L-rhamnose. The MS of taxifolin corresponds to the fragmentation pattern reported for the authentic compound [14]: *m/e* 304 (M), 275, 165, 153, 123, 77, 69. ^{1}H NMR ($(CD_3)_2CO$, 90 MHz, PFT): δ 11.74 (*br s*, 1 H exchangeable with D_2O, C-5-OH), 8.0 (very *br s*, 2 H, exchangeable with D_2O, OH), 7.08 (*s*, 1 H, C-5), 6.89 (*s*, 2 H, C-2′, C-6′), 6.01 (*d*, $J = 1.5$ Hz, 1 H at C-6, AB-system), 5.97 (*d*, $J = 1.5$ Hz, 1 H at C-5, AB-system), 5.03 (*d*, $J = 12$ Hz, 1 H at C-2, AB-system, *trans*-configuration of the coupling protons), 4.61 (*d*, $J = 12$ Hz, 1 H at C-3, AB-system, *trans*-configuration of the coupling protons).

Engelitin (**3**). *U*-10: mp 173–175° (ref. 176–177°). IR (KBr): 3500, 3420 and 3250 (OH), 2950 (CH), 1630, 1580, 1500, 1450, 1350, 1250, 1150, 1040, 970, 830, 816 and 800 cm^{-1}. ^{1}H NMR (CD_3OD, 90 MHz, PFT): δ 1.18 (*d*, $J = 6$ Hz, 3 H from CH_3 of α-L-rhamnose), 4.6 (*d*, $J = 10$ Hz, 1 H at C-3), 5.13 (*d*, $J = 10$ Hz, 1 H at C-2), 5.9 (*d*, $J = 1.5$ Hz, 1 H at C-6), 5.92 (*d*, $J = 1.5$ Hz, 1 H at C-8), 5.82 (*d*, $J = 9$ Hz, 2 H at C-3′, C-5′), 7.34 (*d*, $J = 9$ Hz, 2 H at C-6′).

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