Chemistry of Natural and Microbial Products Dept., National Research Centre, Cairo, Egypt

A new flavone derivative from *Ehretia ovalifolia* leaves

A. M. KHATTAB, M. H. GRACE and E. A. EL-KHRISY

From the methylene chloride extract of *Ehretia ovalifolia* leaves, a new flavone derivative named ovalifolin [3-(3-methyl-1-butenyl)-6-methoxy-5,7,4′-trihydroxy flavone] has been isolated and identified together with the known flavone aglycones apigenin, luteolin and the highly methoxylated flavanol araneosol. The structures of these metabolites have been established on the basis of chemical, chromatographic and spectral methods.

1. Introduction

Ehretia ovalifolia (fam. Boraginaceae) is a deciduous tree distributed from the tropics to the temperate regions of the world [1]. Some species of the genus *Ehretia* were reported to contain pyrrolizidine alkaloids [2], nitrile glucosides, rosmarinic acid with histamine-inhibitory activity [3], dimeric prenylbenzoquinones with antiallergic activity [4] and quinonoid xanthene with antisnake venom activity [5].

Little was reported about the chemical constituents of this species. Eight phenolic compounds have so far been isolated and identified from the bark of *E. ovalifolia*. These are four lignans, a sesquilignan, a neolignan and 2-methoxyhydroquinone glucoside [6].

Here, we report for the first time the isolation and structural elucidation of a new 3-methyl-1-butenyl substituted flavonoid (**1**) and three known flavonoids (**2**–**4**) from the methylene chloride extract of *E. ovalifolia*.

2. Investigations, results and discussion

The dried powdered leaves of *E. ovalifolia* were extracted with 80% EtOH. The concentrated extract was successively partitioned into n-hexane, CH_2Cl_2 and EtOAc-soluble extracts. The CH_2Cl_2 extract, on chromatographic separation on silica gel column, PC and Sephadex LH-20 afforded four flavonoid compounds. On the basis of direct chromatographic comparison with respective reference samples and spectral analyses, compounds **2**–**4** were identified as apigenin, luteolin and araneosol.

Compound **1** was isolated as a faint yellow amorphous powder. Its NaOMe UV spectrum showed a bathochromic shift without decrease in the intensity of band I indicating a free 4′-OH group. The $AlCl_3$, $AlCl_3$/HCl, NaOAc and NaOAc/H_3PO_3 UV spectra exhibited the characteristic features of a free 5, 7, 4′-OH groups [7]. The ^{1}H NMR spectrum of **1** refers to the presence of a 4′- substituted B-ring through a pair of two doublets each integrated to two protons at δ 7.95 ppm (d, J = 8.5) for H-2′, 6′ and 6.95 ppm (d, J=8.5) for H-3′, 5′. A singlet at δ 6.50 ppm is assignable for the proton at C-8. A singlet integrated for three protons at δ 3.80 ppm for one −OMe group suggested to be at position 6. A pair of AB systems with large coupling was observed at δ 6.6 and 5.8 ppm (d, J = 16 Hz) due to the −CH=CH− group of the aliphatic side chain. Further confirmation of the structure was obtained from ^{13}C NMR analysis. The spectrum showed a signal at δ 120.4 ppm for C-3; thus the C-prenylation showed about 15 ppm downfield shift, compared to flavone due to alkyl substitution [8]. The spectrum showed also a C-6 signal at δ 131.6 ppm confirming O-substitution at this position. A signal at δ 94.8 was assignable to the unsubstituted C-8; this value is in the characteristic zone (δ 90–95) of this carbon when it is not substituted [8]. Moreover its EIMS showed two significant ionic peaks at m/z 368 and 353 identified as M^+ and $M-CH_3$ respectively. These results led to the suggestion that the chemical structure of compound **1** is [3-(3-methyl-1-butenyl)-6-methoxy-5,7,4′-trihydroxy flavone]. The new structure was named ovalifolin.

3. Experimental

3.1. Equipment

UV-analyses were run on a Shimadzu UV-Visible spectrophotometer. Analytically pure MeOH was used with each of the shift reagents added separately. ^{1}H NMR and ^{13}C NMR were recorded on a Jeol-EX 270 in $CDCl_3$. The chemical shifts were referred to internal standard TMS. MS were performed on a Finnigan SSQ 7000 mass detector. PC was carried out on Whatmann No. 1 and 3 MM paper using solvent systems a. 15% AcOH, b. n-BuOH-AcOH-H_2O (4 : 1 : 5). TLC was carried out on pre-coated TLC sheets, GF_{254}, solvent system benzene-EtOAC (8 : 2)

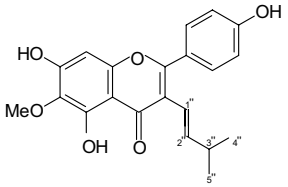
3.2. Plant material

Ehretia ovalifolia leaves were collected in May 1997 from the Orman Botanical Garden and authenticated by Mrs. Terese Labib, Herbarium manager of the Orman Garden.

3.3. Extraction and isolation

The dry powder of *E. ovalifolia* leaves (0.5 kg) was extracted with 80% EtOH and concentrated. The aqueous extract solution was extracted successively with n-hexane, CH_2Cl_2 and EtOAc. The CH_2Cl_2 extract (4 g) was

Table: ^{13}C NMR assignment of compound 1 in $CDCl_3$



C-No.	δ ppm	C-No.	δ ppm
C-2	158.7	C-1′	121.5
C-3	120.4	C-2′	130.2
C-4	181.0	C-3′	115.7
C-5	152.2	C-4′	160.3
C-6	131.6	C-5′	115.7
C-7	155.4	C-6′	130.2
C-8	94.8	C-1″	114.5
C-9	155.7	C-2″	137.0
C-10	104.4	C-3″	38.0
		C-4″	23.0
		C-5″	22.8
		O−CH_3	59.7

chromatographed by vacuum CC over silica gel (Merck, 40–63 μm) and eluted with n-hexane and increasing conc. of EtOAc. A total of 25 frs of 200 ml were collected and combined on the basis of of TLC analysis. Purification was performed by PC using 15% AcOH and BAW (4 : 1 : 5) on Whatmann 3MM. Final purification of each compound for spectral analysis was done over Sephadex LH-20 to yield 20, 30, 15, 25 mg of compounds **1**–**4** respectively.

3.4. Ovalifolin (**1**)

R_f-values (PC): 0.04 (15% AcOH), 0.82 (BAW 4 : 1:5) λ_{max} (MeOH) 270, 336, NaOMe: 275, 329, 395, $AlCl_3$: 277, 304, 350, 382, $AlCl_3$/HCl: 277, 302, 340, 380, NaOAc: 278, 303, 380, NaOAc/H_3BO_3: 274, 345. ^{1}H NMR, δ 7.95 ppm (d, J = 8.5, H-2′, 6′), 6.95 ppm (d, J = 8.5, H-3′, 5′), 6.50 ppm (s, H-8), 6.60 ppm (d, J = 16 Hz, H-1″), 5.80 ppm (d, J = 16, H-2″), 3.80 ppm (OMe group), 0.9–1.4 ppm (7 H; 2 CH_3 + CH) of the aliphatic chain. ^{13}C NMR see Table. EIMS m/z (rel. int.%): 268(75) [M+], 253(100) [M−CH_3], 121(5).

References

1 "Hortus Third", A concise Dictionary of Plants Cultivated in the United States and Canada 1976
2 Suri, O. P.; Jamwal, S. J.; Suri, K. A.; Atal, C. K.: Phytochemistry **19**, 1273 (1980)
3 Simpol, L. R.; Otsuka, H.; Ohtani, K.; Kasai, R.; Yamasuki, K.: Phytochemistry **36**, 91 (1994)
4 Yamamura, S.; Simpol, L. R.; Ozawa, K.; Ohtani, K.; Otsuka, H.; Kasai, R.; Yamasaki, K. and Padolina, W. G.: Phytochemistry **39**, 105 (1995)
5 Selvanayagam, Z. E.; Gnanavendham, S. G.; Balakrishna, K.; Rao, R. B.; Sivaraman, J.; Subramanian, K.; Puri, R.; Puri, R. K.: J. Nat. Prod. **59**, 664 (1996)
6 Yoshikawa, K.; Kageyama, H.; Arihara, S.: Phytochemistry **39**, 659 (1995)
7 Agrawal, P. K.; Thakur, R. S.; Bansal, M. C. in: Agrawal, P. K. (ed): ^{13}C NMR of Flavonoids, Elsevier, New York 1989
8 Mabry, J. T.; Markham, K. R.; Thomas, M. B.: The Systematic Identification of Flavonoids, Springer Verlag, New York 1970

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Dr. Mary H. Grace
Chemistry of Natural
and Microbial Products Dept.
National Research Centre
Cairo
Egypt
mhgrace@thewayout.net