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SHORT REPORTS

A PHENOLIC AMIDE AND OTHER CONSTITUENTS OF *MELANDRIUM FIRMUM*

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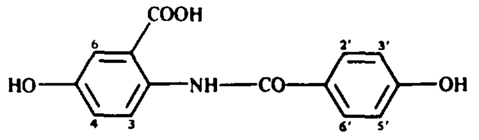
Key Word Index—*Melandrium firmum*; Caryophyllaceae; phenolic acid amide; melandrin; linarin; α-spinasterylglucoside.

Abstract—α-Spinasterylglucoside, linarin and a new phenolic acid amide named melandrin were isolated from *Melandrium firmum* (Caryophyllaceae). The structure was elucidated as *N*-*p*-hydroxybenzoyl-5-hydroxyanthranilic acid on the basis of its spectral data and synthesis.

Melandrium firmum is a herb drug which has been used externally as a vulnerary, styptic and anodyne to treat cuts and wounds, applied to boils and scabies and used internally as a remedy for diseases of lactation, to normalize blood circulation and to treat gonorrhoea, in various parts of the East Asia [1].

In the course of a phytochemical study of this plant, α-spinasteryl-D-glucoside, mp 282–283°, $[\alpha]_D^{16} = -36.2°$, linarin, mp 248–250°, $[\alpha]_D^{20} = -90°$, and a new phenolic acid amide named melandrin were isolated from the ethyl acetate soluble fraction of the methanol extract. Melandrin, $C_{14}H_{11}NO_5$, ($[M]^+$, *m/z* 273, 11.5%) which gave no distinct mp, showed the presence of a hydroxyl group (3200 cm^{-1}), carboxylic acid group (1680 cm^{-1}), acid amide bond (1640, 1540 cm^{-1}) and aromatic ring systems (1600, 1510, 1455 cm^{-1}) in its IR spectrum. ^{1}H NMR of the compound showed two *ortho*-coupled doublets each of two protons with a *J* value of 8.6 Hz at δ7.77 and 6.89, indicating the presence of a 1,4-disubstituted benzene ring and an *ortho*-coupled doublet of one proton with *J* value of 9 Hz at δ8.47, a *meta*-coupled doublet of one proton with *J* value of 3 Hz at δ7.41 and a double doublet of one proton with *J* values of 9 and 3 Hz centred at δ7.03, indicating the presence of a 1,2,4-trisubstituted benzene ring. Three singlets at δ11.6, 10.13 and 9.52 could be assignable to two phenolic protons, a carboxylic proton and an amide proton, respectively. ^{13}C NMR of the compound showed 12 unique types of carbon, whereas the formula of this compound shows 14 carbon atoms. This is due to an element of symmetry, which has already been shown from the ^{1}H NMR. It is clear that the compound has seven non-protonated carbons and seven methine carbons judging from APT spectrum. As expected this compound gave a trimethylated substance, mp 153–154° and a diacetate, mp 118°. Therefore this compound must be a *N*-benzoyl-aniline derivative having two OH groups and one carboxylic acid group.



The appearance of an intense peak at *m/z* 121 ($[HO\,C_6H_4CO]^+$, 100%) in the MS of the compound suggested the presence of a *p*-hydroxybenzoyl group in the compound.

For the 1,2,4-trisubstituted benzene ring there are six possible structures, among which the calculated chemical shift values of ^{1}H NMR [2] and ^{13}C NMR [3] for 2-acylamino-5-hydroxybenzoic acid are almost the same as observed values. Therefore the structure of melandrin was suggested as *N*-*p*-hydroxybenzoyl-5-hydroxyanthranilic acid and this was confirmed by synthesis.

All the spectral data of the synthetic compound are in excellent agreement with those of the natural product. Although chemically similar phenolic acid amides (*N*-salicyl-4-hydroxyanthranilic acids) were identified in other species of the same family (*Dianthus caryophyllus* [4], the occurrence of 5-hydroxyanthranilic acid derivative has not previously been found in the plant kingdom.

EXPERIMENTAL

Plant material. Raised from seeds collected in the wild in the vicinity of Seoul and grown in the experimental garden of the Institute. A voucher specimen is deposited in the herbarium of the Institute.

Isolation of the compounds. Powdered whole plants of *M. firmum* (4.75 kg) were extracted with MeOH. The MeOH extract (190 g) was partitioned with $CHCl_3$ and then EtOAc. The EtOAc phase was concentrated *in vacuo* (10 g) and subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (25:6:0.7) to yield three compounds. *α-Spinasteryl-D-glucoside*, mp 282–283°, colourless needles from MeOH, LB; positive, identified by direct comparison with an authentic sample (IR, MS and co-TLC). Acetate, mp 174–175° (NMR and co-TLC). Acid hydrolysis gave α-spinasteroal (MS and co-TLC) plus glucose

Table 1. ^{13}C NMR data of melandrin (20 MHz, DMSO)

δ	Carbon no.
169.7 (n)	COOH
164.5 (n)	CONH
160.8 (n)	4′
152.5 (n)	5
133.5 (n)	2
129.2	2′,6′
125.7 (n)	1′
122.2	4*
121.5	3*
118.2 (n)	1
117.0	3′,5′
115.7	6

(n) Indicates non-protonated carbon. Others are all methine carbons.

*Interchangeable.

(co-TLC). *Linarin*, mp 248–250°, white powder from MeOH; $FeCl_3$, Mg + HCl, Zn + HCl and Molisch test, positive, identified by direct comparison of an authentic sample (IR, UV, NMR and co-TLC). Acetate, mp 115–117° (NMR and co-TLC). Acid hydrolysis gave acacetin, mp 246–248° (IR, UV, NMR, MS and co-TLC) plus glucose and rhamnose (co-TLC). *Melandrin*, colourless prisms from MeOH; $FeCl_3$, Dragendroff, negative; UV λ_{max}^{MeOH} nm: 336, 290, 237; IR, MS, ^{1}H NMR, see text; ^{13}C NMR, see Table 1.

Methylation of melandrin. Methylation of melandrin (10 mg) with ethereal CH_2N_2 in the usual manner and crystallization from MeOH gave colourless needles, mp 153–154°, MS (*m/z*, rel. int.): 315 ($[M]^+$, 9.6), 135 ($[CH_3O-C_6H_5CO]^+$, 100). ^{1}H NMR ($CDCl_3$); 8.84 (1H, *d*, $J = 9$ Hz, H-3), 7.99 (2H, *d*, $J = 8.8$ Hz, H-2′ and 6′), 7.56 (1H, *d*, $J = 3$ Hz, H-6), 7.04 (1H, *dd*, $J = 3$ and 9 Hz, H-4), 6.99 (2H, *d*, $J = 8.8$ Hz, H-3′ and 5′), 3.95, 3.87. 3.83 (3H each, all *s*, -OMe).

Acetylation of melandrin. Melandrin (5 mg) was treated with Ac_2O (0.5 ml) and pyridine (0.2 ml) for 18 hr and worked up as usual. The reaction product was crystallized from MeOH to give colourless prisms, mp 118°. ^{1}H NMR ($CDCl_3$); 8.32 (1H, *d*, $J = 8.9$ Hz, H-3), 7.95 (1H, *d*, $J = 2.5$ Hz, H-6), 7.71 (2H, *d*, $J = 8.7$ Hz, H-2′ and 6′), 7.45 (1H, *dd*, $J = 2.5$ and 8.9 Hz, H-4), 7.25 (2H, *d*, $J = 8.7$ Hz, H-3′ and 5′), 2.34, 2.32 (3H each, all s, acetate).

Synthesis of melandrin. Melandrin was synthesized by the action of *p*-hydroxybenzoyl chloride in the presence of pyridine on 5-hydroxyanthranilic acid, mp 250° (dec), which was prepared from *m*-hydroxybenzoic acid according to ref. [5]. The synthetic compound was identical to the natural compound in every respect.

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