Phytochemistry, Vol. 24, No. 4, pp. 873–875, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

ASPLENETIN, A FLAVONE AND ITS GLYCOSIDE FROM *LAUNAEA ASPLENIFOLIA*

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(*Revised received* 10 *September* 1984)

Key Word Index—*Launaea asplenifolia* Compositae; asplenetin; asplenin; flavones.

Abstract—A new flavone, asplenetin, has been isolated from *Launaea asplenifolia* and characterized as 5,7,3′,4′,5′-pentahydroxy-3-(3-methylbutyl)flavone. Its glycoside, asplenetin 5-*O*-neohesperidoside, is also reported.

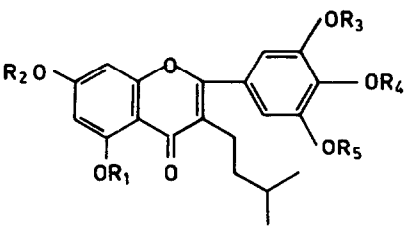
Launaea asplenifolia Hook. (Compositae), a small herb, has been used as a lactagogue in combination with other drugs [1]. No systematic chemical examination has so far been carried out on this plant. We have reported previously, however, the presence of octacosanoic acid, lupeol, 7-hydroxy-3′,4′-dimethoxyflavone, apigenin, luteolin, vitexin, apigenin 7-glucoside, luteolin 7-glucoside and delphinidin from this plant [2]. Now we report the isolation and characterization of a new flavone, asplenetin (**1**), characterized as 5,7,3′,4′,5′-pentahydroxy-3-(3-methylbutyl)flavone, along with its glycoside (**2**) the 5-*O*-neohesperidoside.

The elemental analysis and molecular ion peak at *m/z* 372 in the mass spectrum of **1** led to the molecular formula $C_{20}H_{20}O_7$. Its colour reactions indicated it to be a flavone. The IR spectrum exhibited absorption bands at 1650 and 1600 cm^{-1} due to the presence of an α,β-unsaturated ketonic function. Other peaks, at 3440 cm^{-1} due to polyhydroxyl groups, 2840 and 2920 cm^{-1} due to methyl and CH_2 groups, 1500–1600 cm^{-1} due to the aromatic ring containing phenolic groups and 1100–1300 cm^{-1} due to phenolic groups, were also structurally indicative. A green colour with aqueous ferric chloride indicated the presence of a 5-hydroxyl group [3], which was evidenced by its UV spectrum exhibiting a bathochromic shift of 35 nm in band I in the presence of aluminium chloride–hydrochloric acid [4]. The compound was soluble in aqueous sodium carbonate, providing evidence for the presence of phenolic groups at the 7- and 4′-positions [3]. The presence of a 7-hydroxyl group was supported by a bathochromic shift of 10 nm in band II with sodium acetate relative to its methanol spectrum, while the presence of a 4′-hydroxyl group was confirmed by bathochromic shifts of 50 and 45 nm in band I with sodium methoxide and sodium acetate, respectively [5]. A hypsochromic shift of 22 nm in band I with aluminium chloide–hydrochloric acid relative to its aluminium chloride spectrum indicated the presence of three adjacent hydroxyl groups in the B-ring [5]. The bathochromic shift, by 10 nm in band I with sodium acetate–boric acid, indicated the presence of two hydroxyl groups *ortho* to each other at the 3′- and 4′-positions [5].

The high resolution ^{1}H NMR spectrum of **1** displayed signals at δ0.95 [6H, *d*, J = 6 Hz, $(C\underline{H}_3)_2CH$–], 1.41 [3H, *m*, $(CH_3)_2C\underline{H}C\underline{H}_2$–] and 2.83 (2H, *d*, J = 8 Hz, benzylic-$C\underline{H}_2$) indicating the presence of a 3-methylbutyl side chain. Of the aromatic protons, H-6 and H-8 appeared as doublets (J = 2 Hz) at δ6.15 and 6.45, respectively, whereas H-2′ and H-6′ appeared as a singlet at δ7.35.

Compound **1** formed a penta-acetate (**1a**) which gave no colour with ferric chloride and did not exhibit any absorption band in the 3350–3450 cm^{-1} region in its IR spectrum indicating complete acetylation. However, sharp peaks were observed at 1750 (C=O) and 1200 cm^{-1} (C–O, ester group) in its IR spectrum and a broad signal at δ2.26–2.45 due to overlapping singlets of five acetoxyl groups in its ^{1}H NMR spectrum.

Compound **1**, on alkaline degradation with 50% aqueous potassium hydroxide, afforded gallic acid [5] strongly supporting the presence of three adjacent hydroxyl groups at the 3′-, 4′- and 5′-positions. It was evident, therefore, that the 3-methylbutyl group must be present at C-3, since no singlet due to H-3 could be observed near δ6.3 in its ^{1}H NMR spectrum. This



1 R_1, R_2, R_3, R_4, R_5 = OH

1a R_1, R_2, R_3, R_4, R_5 = OAc

2 R_1 = Neohesperidosyl; R_2, R_3, R_4, R_5 = OH

2a R_1 = OH; R_2, R_3, R_4, R_5 = OMe

2b R_1 = Glucosyl; R_2, R_3, R_4, R_5 = OH

2c R_1 = Neohesperidosyl (6x OAc);
R_2, R_3, R_4, R_5 = OAc

conclusion was further confirmed by its mass spectrum which exhibited a prominent peak at *m/z* 163 establishing the structures of rings B and C [6], while peaks at *m/z* 152 and 153 supported the structure of ring A. Thus, **1** is the 5,7,3′,4′,5′-pentahydroxy-3-(3-methylbutyl)flavone, asplenetin.

Compound **2** also gave colour reactions characteristic of flavones and responded positively to the Molisch test and to reduce Tollens' reagent indicating it to be a flavone glycoside. Its ^{1}H NMR spectrum exhibited signals at δ1.1 (3H, *d*, J = 6 Hz, rhamnosyl CH_3), 3.75 (10H, *m*, rhamnoglucosyl protons), 5.0 (1H, *d*, J = 2 Hz, rhamnosyl H-1) and 5.15 (1H, *d*, J = 7 Hz, glucosyl H-1), indicating the presence of a rhamnoglucosyl moiety in the molecule.

On acid hydrolysis, **2** afforded **1**, glucose and rhamnose. The ratio of the aglycone to the glycoside was found to be 42.4% indicating the presence of 2 mols of sugars/mol of aglycone, i.e. 1 mol each of glucose and rhamnose [7].

It was apparent from the UV spectral data of **1** and **2** that the 5-hydroxyl group in **2** was engaged in the glycosidic linkage. Complete methylation of **2** yielded **2a**. Its ^{1}H NMR spectrum exhibited overlapping singlets at δ3.85 (12H), attributable to four methoxyl groups and a bathochromic shift of 33 nm in band I in its UV spectrum in the presence of aluminium chloride–hydrochloric acid indicating the presence of a free 5-hydroxyl group, confirming that the 5-hydroxyl group in **2** was linked to sugar.

Mild hydrolysis of **2** with 2% sulphuric acid revealed the initial removal of a glucose unit followed by a rhamnose unit [8]. Partial hydrolysis of **2** with formic acid in boiling cyclohexanol afforded a mixture of compounds **1**, **2** and **2b** [9]. Compound **2b** on hydrolysis with β-glucosidase furnished **1** and glucose. These observations and a doublet in ^{1}H NMR spectrum of **2** at δ5.15 (J = 7.0 Hz), due to diaxial coupling of H-1 with H-2 of glucose [5], indicated the β-linkage of a glucose moiety with a flavonoid unit. Further, a doublet at δ5.0 (J = 2 Hz), due to equatorial–equatorial coupling of H-1 with H-2 of the rhamnose unit indicated an α-linkage with the glucose moiety [5].

A three proton doublet at δ1.1 (J = 6 Hz, rhamnosyl CH-3) and two doublets at δ5.0 (J = 2 Hz, rhamnosyl H-1) and 5.15 (J = 7 Hz, glucosyl H-1) in the ^{1}H NMR spectrum of **2** indicated a neohesperidosyl moiety in the molecule [5]. The deca-acetate, **2c**, of **2** displayed no signal in the range δ1.70–1.75 due to the 2-acetoxyl group of the glucose unit, while other aliphatic and aromatic acetoxyl singlets were observed at δ1.95–1.14 (18H, 6 × $-OCOCH_3$) and 2.25–2.45 (12H, 4 × $-OCOCH_3$), respectively. The singlet at δ1.95, assignable to the 6-acetoxyl group of the glucose unit indicated the presence of a free 6-hydroxyl group from the glucose unit in **2** [10]. It was, therefore, concluded that the 2-hydroxyl group of the glucose unit was engaged in the glycosidic linkage with the rhamnose moiety [10]. Thus, **2** is asplenetin 5-*O*-neohesperidoside.

EXPERIMENTAL

The IR spectra were recorded in KBr. The ^{1}H NMR spectra were run at 60 and 90 MHz in DMSO-d_6 and $CDCl_3$ using TMS as int. standard. Elemental analysis was carried out on Hosli's micro-combustion apparatus type CHA.

Isolation of the constituents. The plant material (2.5 kg) collected from Saharanpur region (U.P.) during March was extracted with EtOH. The extract was concd giving a viscous mass (70 g) which was added to H_2O (200 ml) and then extracted successively with petrol and EtOAc. The EtOAc extract was fractionated into $CHCl_3$- and MeOH-soluble portions. The $CHCl_3$ fraction afforded crude **1** on chromatography, while the MeOH-soluble portion yielded **2**. Crude **1** was dissolved in EtOAc and extracted with 5% Na_2CO_3. The alkaline layer was acidified with dil. HCl and digested for 5 min in a water bath. The solid mass obtained was separated, washed with cold H_2O and crystallized from EtOH. Compound **2** was dissolved in hot H_2O. On cooling, a solid mass was obtained. It was separated, washed with cold H_2O and crystallized from EtOH.

Asplenetin (**1**). Yellow-orange crystals (1.5 g) from EtOH, mp 240–243°; R_f: 0.68 (TBA = *t*-BuOH–HOAc–H_2O, 3:1:1), 0.06 (15% HOAc); UV: deep purple; UV–NH_3: yellow; UV λ_{max}^{MeOH} nm: 265, 280 sh, 318, 365; $\lambda_{max}^{MeOH+NaOMe}$ nm: 265, 310 sh, 415, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 268, 318 sh, 355, 422; $\lambda_{max}^{MeOH+AlCl_3-HCl}$ nm: 265, 318 sh, 400; $\lambda_{max}^{MeOH+NaOAc}$ nm: 275, 355 sh, 410; $\lambda_{max}^{MeOH+NaOAc-H_3BO_3}$ nm: 266, 318 sh, 375 nm; ^{1}H NMR (DMSO-d_6): δ0.95 [6H, *d*, J = 6.0 Hz, $(C\underline{H}_3)_2$–CH–], 1.41 [3H, *m*, $(CH_3)_2$–C$\underline{H}$C$\underline{H}_2$], 2.83 (2H, *d*, J = 8 Hz, benzylic–C$\underline{H}_2$–), 6.15 (1H, *d*, J = 2 Hz, H-6), 6.45 (1H, *d*, J = 2 Hz, H-8), 7.35 (2H, *s*, H-2′, H-6′); MS *m/z* (rel. int.): 372 $[M]^+$ (100), 371 (20), 357 (15), 354 (18), 315 (22), 163 (45), 153 (25), 152 (15); (Found: C, 64.23; H, 5.35; $C_{20}H_{20}O_7$ requires: C, 64.52; H, 5.64%.)

Asplenetin penta-acetate (**1a**). Prepared by treating **1** (50 mg) with Ac_2O–pyridine (1:1) giving yellow needles (45 mg) mp 160–163°; IR ν_{max}^{KBr} cm^{-1}: 1750 (C=O), 1200 (C–O ester); ^{1}H NMR ($CDCl_3$): δ2.26–2.45 (15H, *m*, 5 × $-OCOC\underline{H}_3$).

Glycoside (**2**). Yellow-orange crystals from EtOH (2.0 g), mp 172–174°; R_f: 0.28 (TBA), 0.33 (HOAc); UV: deep purple, UV–NH_3: yellow-green; UV: λ_{max}^{MeOH} nm: 268, 280 sh, 318 sh, 365; $\lambda_{max}^{MeOH+NaOMe}$ nm: 260, 280 sh, 410; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 250, 285 sh, 317 sh, 385; $\lambda_{max}^{MeOH+AlCl_3-HCl}$ nm: 250, 280 sh, 318 sh, 365; $\lambda_{max}^{MeOH+NaOAc}$ nm: 383, 320 sh, 375 sh, 405; $\lambda_{max}^{MeOH+NaOAc-H_3BO_3}$ nm: 260, 290 sh, 320 sh, 375; ^{1}H NMR (DMSO-d_6): δ0.96 [6H, *d*, J = 6 Hz, $(C\underline{H}_3)_2$–CH–], 1.45 [3H, *m*, $(CH_3)_2C\underline{H}C\underline{H}_2$–], 2.85 (2H, *d*, J = 8 Hz, benzylic–C$\underline{H}_2$–], 1.1 (3H, *d*, J = 6 Hz, rhamnosyl–C$\underline{H}_3$), 3.75 (10H, *br s*, rhamnoglucosyl protons), 5.0 (1H, *d*, J = 2 Hz, rhamnosyl H-1), 5.15 (1H, *d*, J = 7 Hz, glucosyl H-1), 6.14 (1H, *d*, J = 2 Hz, H-6), 6.45 (1H, *d*, J = 2 Hz, H-8), 7.35 (2H, *s*, H-2′, H-6′).

Partial hydrolysis of asplenin. Compound **2** (50 mg) was added to boiling cyclohexanol (4 ml) and HCO_2H (2 ml). The contents were refluxed at 100–110° for *ca* 10 hr. The hydrolysate was examined by PC which revealed the presence of rhamnose, glucose, asplentin, **2** and **2b**. The **2b** was isolated by PC, dissolved in aq. EtOH (8 ml) and mixed with emulsin (8 ml, isolated from almond seeds). The mixture was refluxed for *ca* 6 hr at 40° and allowed to stand for 72 hr at room temp. The resulting mixture, on extraction with EtOAc furnished **1**, while glucose was detected in the aq. layer.

Acknowledgements—We are grateful to Dr. K. L. Dhar, Assistant Director and Chairman, Natural Products Division (Chemistry), RRL, Jammu and to C.S.I.R., New Delhi for financial assistance to one of us (B.A.).

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Phytochemistry, Vol. 24, No. 4, pp. 875–876, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

FURTHER STUDIES ON THE ISOFLAVONES OF *TEPHROSIA MAXIMA*

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(*Received* 30 *July* 1984)

Key Word Index—*Tephrosia maxima*; Leguminosae; isoflavones; maxima isoflavone B; maxima isoflavone H.

Abstract—A new 7,8-methylenedioxyisoflavone, maxima isoflavone H, was isolated from *Tephrosia maxima* along with the known isoflavone, maxima isoflavone B.

In continuation of our studies on the isoflavones of *Tephrosia maxima* [1], we report here the isolation and characterization of another new isoflavone designated as maxima isoflavone H, in addition to the known isoflavone maxima isoflavone B [2].

An isoflavone fraction obtained by chromatography of the root chloroform extract was found to run close to maxima isoflavones A and B. Although it gave a single spot in TLC, chemical ionization mass spectrometry showed it to be a mixture of three compounds [MS *m/z* (CH_4) (rel. int.): 351 (21), 311 (60.6), 297 (100), 283 (30)]. On coupling, the chemical ionization mass spectrometry with electron impact mass spectrometry and ^{1}H NMR, it was inferred that the substance was a mixture of maxima isoflavones A and B and a new isoflavone having a methylenedioxy substituent.

The mixture was treated with 2 M alcoholic hydrochloric acid [1] and the pure isoflavones isolated by CC of the hydrolysate. Maxima isoflavone A was first eluted along with maxima isoflavone H (both unaffected) followed by pseudobaptigenin (formed from maxima isoflavone B). Maxima isoflavone H was separated from maxima isoflavone A by repeated fractional crystallization.

Maxima isoflavone H gave a positive Labat test and showed carbonyl absorption at 1624 cm^{-1} in its IR spectrum. Its ^{1}H NMR spectrum revealed the presence of one methylenedioxy and one methoxy group. The presence of the methylenedioxy group in ring A and the methoxy group in ring B was evident from the mass spectrum of the compound. As expected [3], its mass spectrum gave $[M]^+$ 296 (100), 164 (83.1) corresponding to the A ring fragment ion, and 132 (27.2) corresponding to the B ring fragment ion. The ^{1}H NMR spectrum of the compound showed the position of the methylenedioxy substituent as 7.8 by its two *ortho* coupled doublets at δ7.87 and 6.94 (J = 9 Hz), each integrating for one proton, which can be assigned to H-5 and H-6, respectively [4]. The presence of the methoxy group at the 4′-position was inferred by the two doublets at δ7.46 and 6.94 (J = 9 Hz) each integrating for two protons. Thus, the structure of maxima isoflavone H was established as 7,8-methylenedioxy-4′-methoxyisoflavone. Maxima isoflavone H represents the third example of 7,8-methylenedioxyisoflavones, the first two being maxima isoflavone A [5] and maxima isoflavone D [1].

Maxima isoflavone B, the structure of which was established earlier as 7-γ,γ-dimethylallyloxy-3′,4′-methylenedioxyisoflavone by chemical degradation [6] and synthesis [7], has now been characterized by spectral data.

EXPERIMENTAL

Refer to our earlier paper [1] for plant material employed and extraction details. CC of the root $CHCl_3$ extract (24 g) yielded maxima isoflavone B (127 mg, R_f 0.78 in 5% Me_2CO in benzene) and maxima isoflavone H (54 mg, R_f 0.75 in 5% Me_2CO in benzene), apart from the previously reported isoflavones.

Maxima isoflavone B. Colourless needles, mp 126–128° identical with lit. [6] value (126–128°); UV λ_{max}^{MeOH} nm: 218, 252, 298; IR ν_{max}^{KBr} cm^{-1}: 1635, 1490, 1440, 925, 820, 782; ^{1}H NMR (90 MHz, $CDCl_3$): δ7.73 (1H, *s*, H-2), 8.04 (1H, *d*, J = 9 Hz, H-5), 6.70 (1H, *dd*, J = 9, 2 Hz, H-6), 6.95 (1H, *d*, J = 2 Hz, H-8), 6.8–7.2 (3H, *m*, H-2′, H-5′, H-6′), 5.9 (2H, *s*, $-OCH_2O-$), 4.53 (2H, *d*, H-1″), 5.38 (1H, *t*, H-2″), 1.75, 1.80 (6H, two *s*, CH_3-4″, CH_3-5″); MS *m/z* (rel. int.): 350 $[M]^+$ (10.2), 282 $[M - C_5H_8]^+$ (100), 146