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A PRENYLATED FLAVANONE FROM ROOTS OF *AMORPHA FRUTICOSA*

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Key Word Index—*Amorpha fruticosa*; Leguminosae; roots; 5,7,3′-trihydroxy-6,8,5′-triisoprenyl-4′-methoxyflavanone; isoamoritin.

Abstract—From the roots of *Amorpha fruticosa*, a new prenylated flavanone was isolated, in addition to seven known phenolic compounds. The structure of the new flavanone was confirmed to be 5,7,3′-trihydroxy-6,8,5′-triisoprenyl-4′-methoxyflavanone (isoamoritin) by spectroscopic analysis, including 2D NMR. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of studies on phenolic compounds in leguminous plants, we have characterized some interesting prenylated flavanones possessing biological activities [1, 2]. Our search for biological principles from the leguminous plants was continued by examination of the root constituents of *Amorpha fruticosa*. Many previous studies of these plants have revealed the presence of prenylated flavanones [3–5], rotenoids [6–9] and stilbenoids [10]. Biological activities were also mentioned [8].

RESULTS AND DISCUSSION

Dried and powdered roots of *A. fruticosa* were extracted with acetone at room temperature. The acetone extract was subjected to column chromatography over silica gel and eluted with a cyclohexane-acetone mixture. Further purification by column chromatography on silica gel, Sephadex LH20 and vacuum liquid chromatography and preparative TLC resulted in the isolation of eight compounds (**1**–**8**) including a new prenylated flavanone (**1**).

Compound **1** was positive to $FeCl_3$ test on TLC. The HREIMS gave a $[M]^+$ at *m/z* 506.2672 (Calcd: 506.2668) which corresponds to $C_{31}H_{38}O_6$. In the ^1H NMR spectrum, three one-proton double doublet were observed at δ 5.40 (J = 12.2, 3.4 Hz), 2.79 (J = 17.1, 3.4 Hz) and 3.07 (J = 17.1, 12.2 Hz) which were assigned to H-2 and H-3 in a flavanone skeleton. The spectrum also showed the presence of three isoprenyls (δ 1.64 (6H, *br s*, Me × 2), 1.65, 1.72, 1.73, 1.75 (3H each, *br s*, Me), 3.32 (4H, *m*, CH_2 × 2), 3.36 (2H, *d*, J = 7.3 Hz, CH_2), 5.17 (2H, *m*, CH= × 2), 5.29 (1H, *t*-like *m*, CH=), a methoxyl (δ 3.79), three hydroxyl groups [δ 8.11 (2H, *br s*), 12.45 (1H, *s*, chelated)], in addition to a set of two *meta*-coupled protons observed as a broad singlet (δ 6.86 and 6.94). Two significant fragment ions appeared at *m/z* 289 [A_1 + H] and 218 [B_1] in the EI mass spectrum (Scheme 1), indicating that two hydroxyl and two isoprenyl groups were located on the A ring, whilst a hydroxyl, a methoxyl and an isoprenyl group were on the B ring. In the CH COSY spectrum, two overlapped methylene protons at δ 3.32 were correlated to two carbons at δ 21.2 and 22.4, which suggested that both *ortho*-positions of the two isoprenyl groups were substituted with an oxygen function [11]. The chemical shift based on the methylene carbon of the remaining isoprenyl group was observed at δ 28.2, which indicated that one of *ortho*-positions of the isoprenyl group was substituted with an oxygen function [11]. The methoxyl carbon observed at δ 61.2 showed that both *ortho*-positions were occupied with substituents. These results suggested that the A ring had 5,7-dihydroxy-6,8-diisoprenyl substitution and the B ring 3′-hydroxy-4′-methoxy-5′-isoprenyl substitution. Therefore, the structure of **1** is 5,7,3′-trihydroxy-6,8,5′-triisoprenyl-4′-methoxyflavanone and is named isoamoritin. Its structure was substantiated by a COLOC spectrum (Scheme 2 and corresponds to an isomer of amoritin (**9**) [4]. As the flavanone (**1**) has $[\alpha]_D$ −33°, the configuration at C-2 is *S*. Compounds **2**–**8** were identified as amorin (**2**), isoamorin (**3**) [12], 12a-hydroxyamorphigenin (**4**) [9], amorphaquinone (**5**) [12], demethylmedicalpin (**6**), formononetin (**7**) and calycosin (**8**), respectively. All compounds have already

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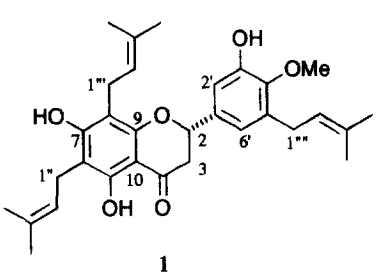
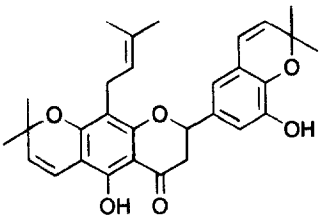


1



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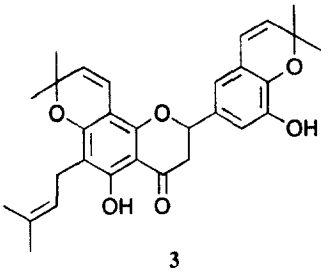

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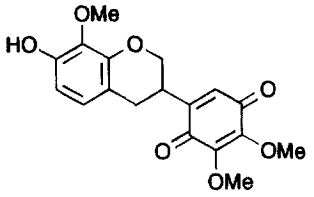

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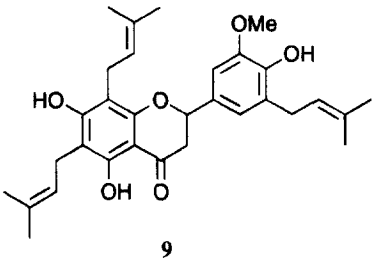

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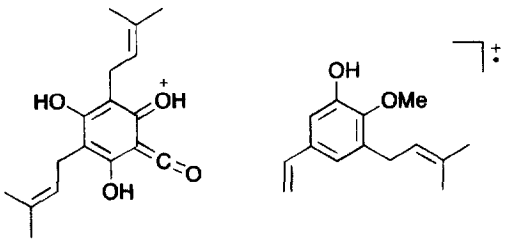
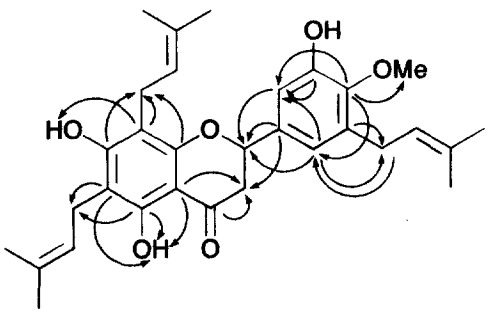


m/z 289 $[A_1+H]^+$ *m/z* 218 $[B_1]^+$

Scheme. 1. EI mass spectral fragments of compound **1**.



Scheme. 2. ^{13}C-^1H long-range correlations in the COLOC spectrum (J = 10 Hz) of compound **1**.

been isolated from different parts of *A. fruticosa*. Amorphaquinone (**5**) has a unique B ring substitution and was first isolated by Shibata *et al.* [13]. The complete assignment of the ^1H NMR spectrum is given in the Experimental.

EXPERIMENTAL

Plant material

Roots of *A. fruticosa* L. were collected in May, 1996 at Motosu district, Gifu Japan. Voucher specimens are deposited at the Herbarium of Gifu Pharmaceutical University.

Isolation of 1–8

Dried and powdered roots (1.2 kg) were extracted with Me_2CO at room temp. and the solvent was conc *in vacuo*. The Me_2CO extract (20 g) was subjected to silica gel CC eluted with a cyclohexane-Me_2CO gradient. The (5:1) and (3:1) frs obtained were combined and further purified by silica gel CC ($CHCl_3$–MeOH mixts), vacuum liquid chromatography (same solvent system as silica gel CC). Sephadex LH20 CC (MeOH and Me_2CO) and prep. TLC to give **1** (21 mg), **2** (13 mg), **3** (10 mg), **4** (12 mg), **5** (133 mg), **6** (5 mg), **7** (4 mg) and **8** (9 mg), respectively.

Compound **1** (*isoamoritin*). Pale yellow solid. $[\alpha]_D$ −33° (*c* 0.2, MeOH). HREIMS: *m/z* 506.2672 for $C_{31}H_{38}O_6$ (Calcd. 506.2668). EIMS *m/z* (rel. int.): 506 (100), 491 (18), 463 (9), 451 (26), 435 (25), 407 (9), 395 (12), 289 (6), 288 (7), 280 (10), 273 (19), 260 (14), 245 (17), 233 (26), 231 (22), 218 (22), 203 (14), 189 (39), 177 (22). UV (nm, MeOH): 225, 292, 335 *sh*. ^1H NMR (400 Mz, acetone-d_6): δ 1.64 (6H, *br s*, Me × 2), 1.65, 1.72, 1.73, 1.75 (3H each, *br s*, Me), 2.79 (1H, *dd*, J = 17.1, 3.4 Hz, H-3*eq*), 3.07 (1H, *dd*, J = 17.1, 12.2, H-3*ax*), 3.32 (4H, *m*, CH_2 × 2, H-1″,1‴), 3.36 (2H, *br d*, J = 7.3 Hz, CH_2, H-1⁗), 3.79 (3H, *s*, OMe), 5.17 (2H, *m*, CH= × 2, H-2″,2‴), 5.29 (1H, *t*-like *m*, CH=, H-2⁗), 5.40 (1H, *dd*, J = 12.2, 3.4 Hz, H-2), 6.86 (1H, *br s*, H-6′), 6.94 (1H, *br s*, H-2′), 8.11 (2H, *br s*, OH × 2, OH-7, 3′), 12.45 (1H, *s*, C-5-OH). (400 MHz, $CDCl_3$): δ 1.73 (9H, *br s*, Me × 3), 1.75 (6H, *br s*, Me × 2), 1.81 (3H, *br s*, Me), 2.79 (1H, *dd*, J = 17.1, 2.9 Hz, H-3*eq*), 2.99 (1H, *dd*, J = 17.1, 13.2 Hz, H-3*ax*), 3.32 (4H, *t*-like *m*, CH_2 × 2, H-1″,1‴), 3.37 (2H, *br d*, J = 7.3 Hz, CH_2, H-1⁗), 3.81 (3H, *s*, OMe), 5.23 (3H, *m*, H-2″,2‴,2⁗), 5.27 (1H, *dd*, J = 13.2, 2.9 Hz, H-2), 6.37

Table 1. ^{13}C NMR spectral data of compound **1** (100 MHz, acetone-d_6)

No.		No.	
2	78.4	1″	21.2
3	43.3	2″	122.1*
4	196.4	3″	134.6†
5	159.3	4″	25.8‡
6	107.3	5″	17.8§
7	157.6	1‴	22.4
8	106.4	2‴	128.8*
9	162.3	3‴	134.0†
10	102.8	4‴	25.8‡
1′	135.6	5‴	17.8§
2′	111.1	1⁗	28.2
3′	149.1	2⁗	122.0*
4′	145.0	3⁗	133.2†
5′	135.0	4⁗	25.8‡
6′	118.8	5⁗	17.8§
		OMe	61.2

*,† interchangeable, ‡,§ overlapping.

(1H, *br s*, OH), 6.75 (1H, *br s*, H-6′), 6.91 (1H, *br s*, H-2′), 12.31 (1H, *s*, C-5-OH). ^{13}C NMR: Table 1.

Compound **5** (*amorphaquinone*). Reddish solid. ^1H NMR (400 MHz, acetone-d_6): δ 2.80 (1H, *dd*, J = 15.6, 8.8 Hz, H-2*ax*), 2.96 (1H, *dd*, J = 15.6, 5.6 Hz, H-2*eq*), 3.37 (1H, *m*, H-3), 3.75, 3.97, 3.98 (3H each, *s*, OMe) 4.06 (1H, *dd*, J = 10.5, 7.6 Hz, H-4*ax*), 4.34 (1H, *br d*, J = 10.5 Hz, H-4*eq*), 6.42 (1H, *s*, H-6′), 6.43 (1H, *d*, J = 8.3 Hz, H-6), 6.67 (1H, *d*, J = 8.3 Hz, H-5), 7.67 (1H, *s*, OH). ^{13}C NMR (100 MHz, acetone-d_6): δ 29.8 (*t*, C-4), 31.6 (*d*, C-3), 60.6, 61.2, 61.4 (*q*, OMe), 69.0 (*t*, C-2), 109.1 (*d*, C-6), 113.8 (*s*, C-10), 124.7 (*d*, C-5), 131.5 (*d*, C-6′), 136.5 (*s*, C-1′), 145.7, 146.3, 147.3, 148.3, 138.7 (*s*, C-7-9, 3′,4′), 184.0, 184.5 (*s*, C-2′,5′).

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