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AN ISOPRENYLATED FLAVANONE FROM LEAVES OF *AZADIRACHTA INDICA**

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(*Revised received* 28 *February* 1984)

Key Word Index—*Azadirachta indica*; Meliaceae; neem; leaf; isoprenylated flavanone; nimbaflavone.

Abstract—A new isoprenylated flavanone has been isolated from the leaves of *Azadirachta indica* and characterized as 8,3′-di-isoprenyl-5,7-dihydroxy-4′-methoxyflavanone on the basis of physical and spectroscopic evidence. This is the first report of an isoprenyl flavanone from the Meliaceae.

Confirmation of hypotensive activity in the chloroform soluble fraction of the ethanolic extract of the leaves of *Azadirachta indica* A. Juss prompted us to undertake a detailed chemical investigation of this fraction. This resulted in the isolation of a new isoprenylated flavanone, nimbaflavone (**2**), the known meliacins [1] nimbolide (**1**) and 3-desacetyl salannin (**3**) and sitosterol. Compound **2** is a minor constituent of the biologically active chloroform soluble fraction.

Compound **2** analysed for $C_{26}H_{30}O_5$ (M^+ 422 *m/z*), $[\alpha]_D^{23°}$ −196° (*c* 0.05; MeOH). The IR spectrum showed the presence of a chelated carbonyl (1640 cm^{-1}) and the aromatic nature of the compound (1620, 1510 cm^{-1}) along with phenolic hydroxyl absorption at 3400 cm^{-1}. It gave a blue colour with ferric chloride and a positive

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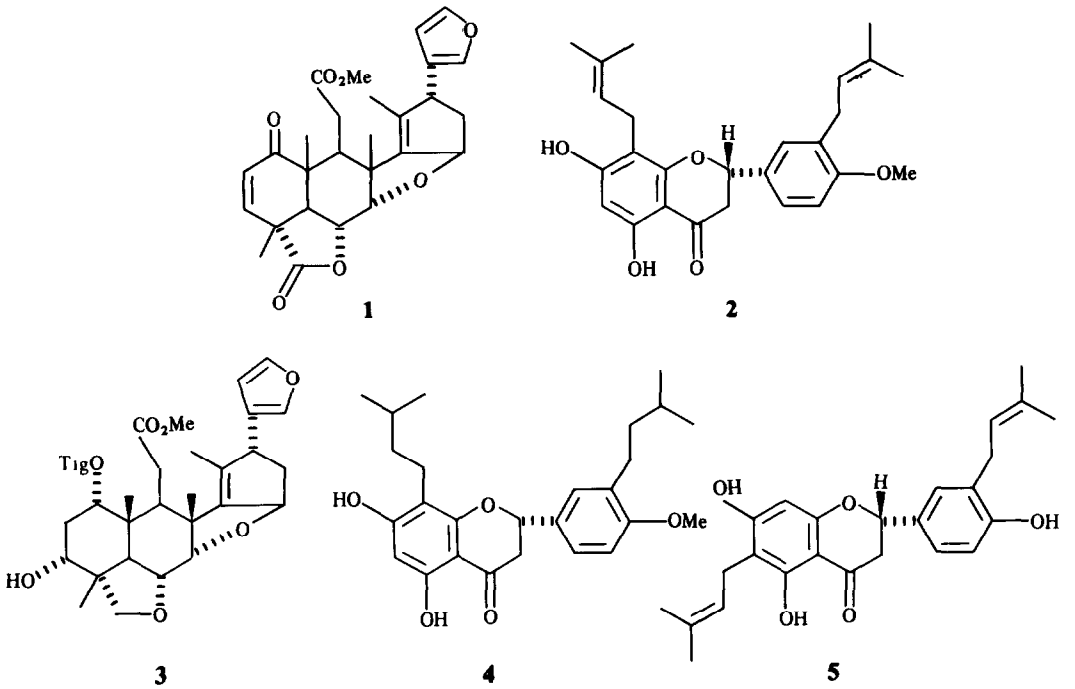
Table 1. UV spectral data for nimbaflavone (**2**) and euchrestaflavone (**5**)

Compound	MeOH max (nm)	+ $AlCl_3$ (nm)	+ $AlCl_3$–HCl (nm)	NaOAc (nm)	NaOMe (nm)
Nimbaflavone (**2**)	290,335 (sh)	313,360	313,360	290,330	290,330
Tetrahydronimbaflavone (**4**)	290,330 (sh)	312,360	310,360	290,330	290,330
Euchrestaflavone* (**5**)	295,340 (sh)	297,340 (sh) and 370	—	295,338	252,337

*UV data of euchrestaflavone are taken from ref. [2].



flavanone reaction with magnesium–hydrochloric acid (red colour). The UV spectrum of λ_{max}^{MeOH} 290, 355 (sh) nm also suggested that it was a flavanone. UV shifts (Table 1) with aluminium chloride, sodium acetate and sodium methoxide confirmed the presence of a 5,7-dihydroxy system in **2**.

In the ^{1}H NMR spectrum of **2** the singlet at δ3.7 was for a methoxymethyl function which could be placed at C-4'. Four olefinic methyls of the two isoprenyl (*v*, *v*-di-methyl allyl) moieties appeared at δ1.67–1.72 and two olefinic protons at δ5.1 and 5.2 were present each as a diffused triplet (J = 2.5 Hz); while the four protons of the two methylenes (Ar–C<u>H</u>$_2$–) of the isoprenyl groups appeared as multiplets at δ3.2. The C-2 proton signal of the flavanone moiety at δ5.3 (*d*, *d*, J = 10 Hz and 4 Hz), was partly masked by olefinic protons. The C-3 protons also as multiplets, appeared at δ2.85. The C-2 proton which gave a double doublet was more pronounced in tetrahydro-nimbaflavone (**4**) obtained from **2** by hydrogenation. The decoupling experiments carried out on both **2** and **4** further confirmed the axial–axial (J = 10 Hz) and axial–equatorial (J = 4.0 Hz) couplings between the C-2 proton and the C-3 protons. The ^{1}H NMR spectrum of **2** in addition showed a lone Ar–<u>H</u> at δ5.85. This aromatic proton could be at C-6 or C-8 in ring-A. Since **2** failed to give a positive Gibb's test, the aromatic H must therefore, be at the C-6 position and position C-8 occupied by an isoprenyl group.

Comparison of the ^{1}H NMR spectrum of **2** and the known isoprenylated flavanone, euchrestaflavone [2] (**5**) revealed that the substitution pattern in the B-ring in these compounds is identical. The ABX system of ring-B (comprising the protons at C-5', C-6' and C-2') in both compounds showed the presence of an *ortho* coupled H-5' at δ6.85 (*d*, J = 10 Hz) and H-2' and H-6' as multiplets between δ7.1–7.2.

The ^{1}H NMR data discussed so far suggested that **2**, like **5**, has an isoprenyl chain attached at C-3' and a methoxy group at C-4' (hydroxyl group in case of **5**). Compounds **5** and **2** are thus isomers and differ only in the placement of an isoprenyl side chain in ring-A. The position of this chain at C-8 in **2** was fixed as follows. Compound **2** did not give a positive Gibb's test. Further, it showed a bathochromic shift of 23 nm of band-II (Table 1) in the UV on the addition of aluminium chloride. The isomeric flavanone, **5**, which has an isoprenyl side chain at C-6 gave a positive Gibb's test and gave no shift in its UV spectrum with aluminium chloride (Table 1). This is in agreement with the observations of Sharif *et al.* [3] who have shown that an aluminium chloride induced shift in the UV spectra of 5-hydroxyflavones in general only occurs in the absence of an alkyl substitution at C-6. The structure **2** assigned to nimbaflavone was in complete agreement.

The structure of **2** was further supported by its mass spectrum and that of its tetrahydro derivative **4** (Fig. 1.). The mass spectrum of **2** showed a molecular ion at *m/z* 422 $[M]^+$. The retro-Diels–Alder fragmentation gave ion **a** at *m/z* 220 constituting ring-A and ion **b** at *m/z* 202 comprising of ring-B. The presence of an isoprenyl side chain in ring-A and ring-B was supported by the presence of ion **c** at *m/z* 165 by the loss of a C_4H_7 unit from ion **a** and ion **d** at *m/z* 147 arising out of ion **b** after a similar loss (C_4H_7). The corresponding ions **a** and **b** in the mass spectrum of **4** appeared at *m/z* 222 and *m/z* 204, respectively; the ion **a** in **4** was of a very low intensity (5%) as it underwent benzylic cleavage to give ion **c** (*m/z* 165, 80%). Other significant ions in the mass spectrum of **2** were at *m/z* 205 (loss of methyl from ion **a**) due to ion **e** and at *m/z* 187 (loss of methyl from ion **b**) due to ion **f**.

Nimbaflavone was thus fully characterised as 8,3'-di-isoprenyl-5,7-dihydroxy-4'-methoxyflavanone (**2**).

Isoprenylated flavanones [4] of the nimbaflavone (**2**) type which exhibit (−) rotation have been assigned '*S*'

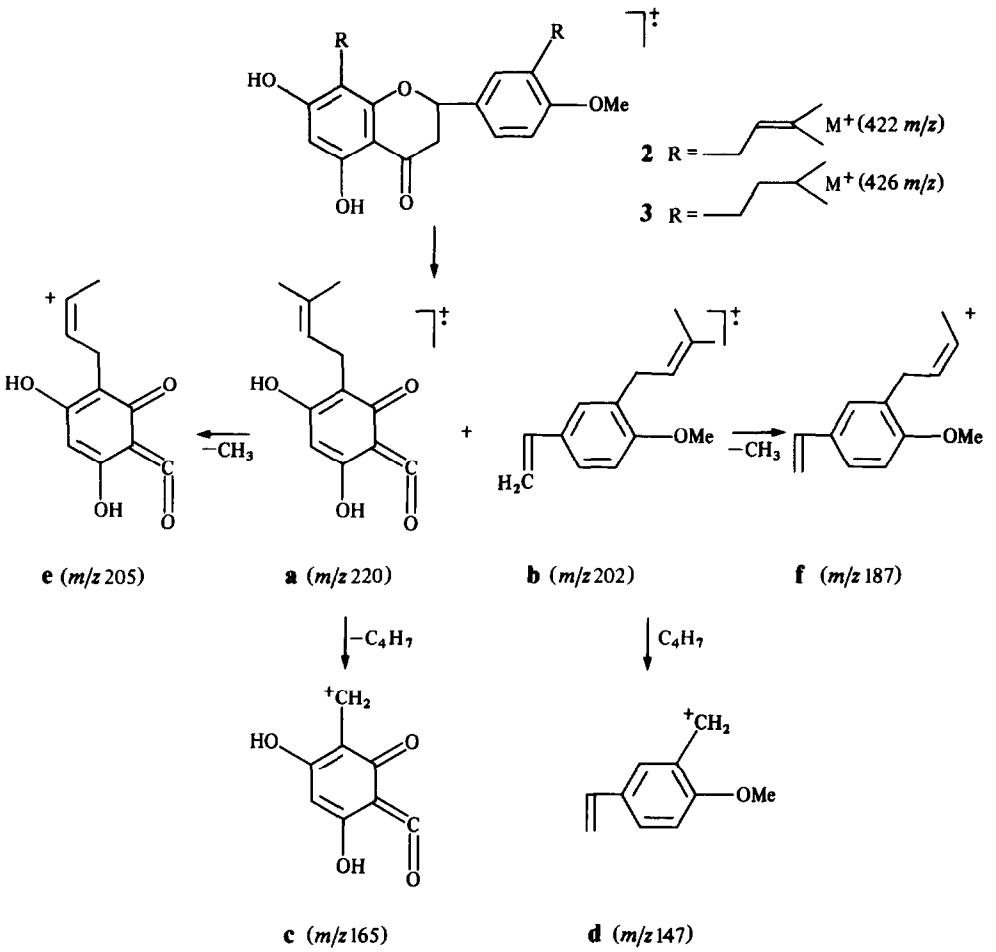


Fig. 1.

configuration at C-2. On similar reasoning **2** should have '*S*' configuration at C-2.

The plants belonging to Meliaceae are generally a rich source of limonoids. Simple flavonoids such as kaempferol and quercetin which occur commonly in plants of Rutaceae have also been isolated from the flowers of *Azadirachta indica* [5]. The isolation of these flavonoids indicate the taxonomic closeness of Meliaceae and Rutaceae. Rutaceous plants in addition have also been known to synthesize isoprenylated flavonoids [4]. Thus the characterisation of an isoprenylated flavanone from *A. indica* further strengthens the taxonomic closeness of the Meliaceae with the Rutaceae.

EXPERIMENTAL

Mps are uncorr. IR: KBr, ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as int. reference on 90 MHz attached with decoupler. TLC was performed on silica gel plates using ceric sulphate as spray.

Isolation. The air-dried leaves (8 kg) of *Azadirachta indica* A. Juss collected locally (identified by Dr. B. N. Mehrotra; voucher specimen deposited in the herbarium of the Institute) were extracted with 90% EtOH at room temp. The EtOH extract was fractionated with hexane, $CHCl_3$ and *n*-BuOH. The $CHCl_3$ soluble fraction (40 g) was chromatographed over silica gel (1.0 kg) and successively eluted with C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH (solvent mix gradient; TLC control) and finally with MeOH resulting in the isolation of the following compounds.

Nimbolide (**1**). The C_6H_6 fractions were concd and the residue crystallised (MeOH) to give a compound with mp 243–245°, $[\alpha]_D^{23°}$ +188° which was found to be identical (mp, IR, UV and ^{1}H NMR) with nimbolide (**1**) [1].

Sitosterol. The fractions eluted with C_6H_6–$CHCl_3$ (4:1) yielded sitosterol, mp 132–134°, $[\alpha]_D^{23°}$ −36° and identical (mp, IR, ^{1}H NMR) with an authentic marker.

Nimbaflavone (**2**). The C_6H_6–$CHCl_3$ (1:1) fraction afforded a mixture of **2** and **3** which was further purified on a silica gel column and eluted with C_6H_6–$CHCl_3$ (2:1 to 1:1) monitored with TLC (C_6H_6–MeOH; 19:1) to give pure **2** as colourless crystals (150 mg) mp 126–128°, $[\alpha]_D^{23°}$ −196° (*c* 0.05, MeOH). IR ν_{max} cm^{-1}: 3400, 1640, 1620, 1510 etc.; UV λ_{max} (nm): 290, 355 (sh), ^{1}H NMR: δ1.67–1.72. (12H, *m*, $2\times\underline{CH_3}>\underline{CH_3}>=\!\!<$Ar), 2.85 (2H, *m*, H-3), 3.2 (4H, *m*, Ar–C$\underline{H_2}$), 3.7 (3H, *s*, OMe), 5.1 (1H, *t*, *J* = 2.5 Hz), 5.2 (1H, *t*, *J* = 2.5 Hz, $>=<_{\underline{CH_2}-Ar}^{\underline{H}}$), 5.3 (1H, *d*, *d*, *J* = 10 Hz and 4.0 Hz), 5.85 (1H, *s*, Ar–$\underline{H}$), 6.85 (1H, *d*, *J* = 10 Hz, H-5'), 7.1–7.25 (2H, *m*, H-2' and H-6'). MS *m/z* (rel. int.): 422 $[M]^+$ (80), 407 $[M-Me]^+$ (10), 369 $[M-C_4H_7]^+$ (20), 220

(ion **a**) (45), 205 (ion **e**) (55), 202 (ion **b**) (30), 187 (ion **f**) (25), 165 (ion **c**) (52), 147 (ion **d**) (30) and 69 $[C_5H_9]^+$ (100).

Tetrahydronimbaflavone (**4**). Compound **2** (50 mg) in MeOH (20 ml) was stirred with Pd–C (10 mg) in presence of H_2 gas at room temp. for 6 hr, filtered and processed to yield **4** (50 mg), mp 130°, $C_{26}H_{34}O_5$ (M^+ 426 *m/z*); IR ν_{max} cm^{-1}: 3338, 3000, 1650, 1620, 1510, 1460, 1380, 1276, 1220, 1140, 1090 and 840. ^1H NMR: δ0.8, 0.85, 0.9, 0.95 (4 × Me), 1.2–1.4 (4H, *m*, side chain methylenes), 2.5 (2H, *m*, methine), 3.7 (*s*, OMe), 5.2 (1H, *d*, *d*, *J* = 10 Hz and 4 Hz H-2), 5.85 (1H, *s*, Ar-H-6), 6.7 (1H, *d*, *J* = 10 Hz, H-5'), 7.1–7.25 (2H, *m*, H-2' and H-6'). MS *m/z* (rel. int.): 426 $[M]^+$ (55), 369 $[M - C_4H_9]^+$ (50), 222 (ion **a**) (~ 5), 204 (ion **b**) (45), 165 (ion **c**) (80), 147 (ion **d**) (30).

3-*Deacetyl salannin* (**3**). The fractions eluted with C_6H_6–$CHCl_3$ (1:2) and $CHCl_3$ (monitored TLC) on removal of solvent yielded a tetranortriterpenoid (250 mg), mp 195–200°; which was found to be identical (mp, IR, ^1H NMR and MS [6] with 3-deacetyl salannin (**3**).

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CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS NUMMULARIA*

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Key Word Index—*Zizyphus nummularia*; Rhamnaceae; peptide alkaloids; nummularine-M; nummularine-N, nummularine-B.

Abstract—In addition to the known alkaloid nummularine-B, two new peptide alkaloids nummularine-M and nummularine-N have been isolated from *Zizyphus nummularia* and their structures elucidated. Nummularine-M is a 14-membered cyclopeptide and belongs to the integerrinine type, whereas nummularine-N is a 13-membered cyclopeptide like nummularine-B.

INTRODUCTION

In continuation of our work on cyclopeptide alkaloids from the plants belonging to the Rhamnaceae we now report the isolation of two new cyclopeptide alkaloids from *Zizyphus nummularia*. About a dozen cyclopeptide alkaloids have so far been reported from the root bark of *Z. nummularia* [1–3]. We report here the alkaloids of the stem bark of this plant which has not yet been investigated. Repeated column chromatography of the alkaloid fraction on silica gel followed by prep. TLC gave small amounts of two new cyclopeptide alkaloids, nummularine-M [4] and nummularine-N along with a known peptide alkaloid, nummularine-B [1].

RESULTS AND DISCUSSION

Nummularine-M, $C_{31}H_{42}N_4O_4$ ($[M]^+$ *m/z* 534.3190) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum [5]. The IR spectrum exhibited bands for –NH, –NMe, –NH–CO and Ar–O–C. It is isomeric with integerrinine (**4**) [5] and both molecules show identical mass fragmentation patterns indicating their gross structural similarity. However, acid hydrolysis revealed the essential difference between the two molecules. Thus, while integerrinine yields *N*,*N*-dimethylisoleucine and leucine on acid hydrolysis, nummularine-M gives *N*,*N*-dimethylisoleucine and isoleucine. Based on these findings the structure of