130.0 (C-1) 129.44 (C-2), 120.1 (C-3), 119.2 (C-1a), 116.49 (C-11a), 101.2 (C-4), 94.5 (C-8), 75.5 (C-6a), 65.0 (C-12a), 62.2 (C-6), 59.0 (OMe); EIMS *m/z* (rel. int.): 330 (40), 312 $[M-H_2O]^+$ (12), 298(5), 183(100), 182(90), 168(60), 167(50), 155(7), 149(10), 148(60), 147(90) etc.

Acetylation (Ac_2O-4-dimethylaminopyridine) gave a triacetate (**2**), mp 206°, R_f 0.66 (hexane–Me_2CO, 3:2), $C_{23}H_{20}O_{10}$. ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.88, 2.1 and 2.3 (9H, 3*s*, 3 × OCOMe), 3.85 (1H, *s*, OMe), 4.39 to 4.8 (3H, *m*, H-6_{ax}H-6_{eq} and H 6a), 6.38 (1H, *s*, H-8), 6.80 (1H, *dd*, $J=8$, 2 Hz, H-4), 7.0 to 7.25 (2H, *m*, H-2 and H-3), 8.35 (1H, *dd*, $J=8$, 2 Hz, H-1).

1 on acid catalysed dehydration with methanolic-HCl for 8 hr at 100° and work-up in usual manner gave **3** (crystals from MeOH), mp 206°, R_f 0.64 (hexane–Me_2CO, 3:2), $C_{17}H_{12}O_6$. UV λ_{max}^{MeOH} nm: 216, 280, 340; +$AlCl_3$ 216, 288, 330; IR ν_{max}^{KBr} cm^{-1}: 3200, 1640, 1520, 1480, 1460, 1400, 1310, 1280, 1220, 1200, 1150, 1000 etc.; ^{1}H NMR (Me_2CO-d_6): δ 3.90 (3H, *s*, OMe), 5.10 (2H, *s*, C-6), 6.50 (1H, *s*, H-8), 7.00 (1H, *dd*, $J=8$, 2 Hz, H-4), 7.25 to 7.50 (2H, *m*, H-2 and H-3), 8.65 (1H, *dd*, $J=8$, 2 Hz, H-1); EIMS *m/z* (rel. int.): 312 (100), 297(60), 284(7), 269(40), 183(8), 182(10), 168(15), 149(70), 130(10), 129(11) etc.

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A BIFLAVONOID FROM *GARCINIA NERVOSA*

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Key Word Index—*Garcinia nervosa*; Guttiferae; new biflavonoid; flavanonylflavone; I-5, II-5, I-7, II-7, I-3′, I-4′, II-4′-heptahydroxy-[I-3, II-8]-flavanonylflavone; structural elucidation.

Abstract—A new biflavonoid has been isolated from the leaves of *Garcinia nervosa*. Its structure I-5, II-5, I-7, II-7, I-3′, I-4′, II-4′-heptahydroxy-[I-3, II-8]-flavanonylflavone was elucidated with the help of chemical and spectral methods.

INTRODUCTION

The genus *Garcinia* consisting of 180 species has been the subject of considerable phytochemical investigations due to the interesting biological properties of many of its species [1, 2]. The genus has been a major source of prenylated xanthones [3], benzophenones [4] and biflavonoids mainly with a 3/8-linkage [5]. Work on the stem bark of *G. nervosa* has recently been performed and a new xanthone named nervosaxanthone has been isolated [6]; however, no biflavonoid could be isolated. We have now investigated the ether soluble part of the alcoholic extract of leaves of *G. nervosa* and report on the isolation of a new biflavonoid characterised with the help of chemical and spectroscopic methods as I-5, II-5, I-7, II-7, I-3′, I-4′, II-4′-heptahydroxy-[I-3, II-8]-flavanonylflavone (**1**).

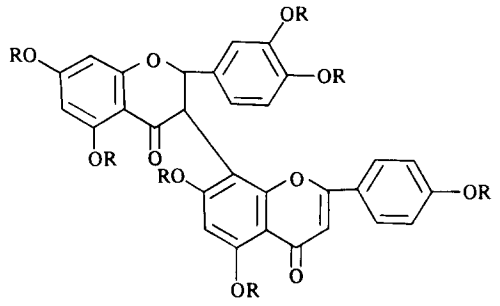
RESULTS AND DISCUSSION

The diethyl ether soluble portion of the alcoholic extract was subjected to CC over silica gel. Elution with benzene–ethyl acetate (2:3) afforded crude **1** which was purified by preparative TLC and crystallized from benzene–methanol as yellow crystals, mp 232–234°.

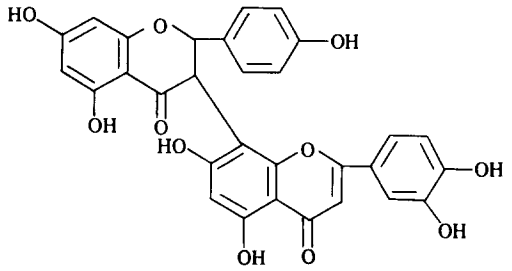
Compound **1** analysing for $C_{30}H_{20}O_{11}$ gave a positive ferric chloride test and a pink colour with Zn–HCl and Mg–HCl suggesting that it was a hydroxylated flavonoid derivative. Treatment of **1** with dimethyl sulphate and potassium carbonate in acetone furnished a heptamethyl ether, mp 123–125°. The IR spectrum of **1** exhibited absorptions at 3300 (OH), 1690 (5-hydroxyflavanone) and 1610 cm^{-1} (5-hydroxyflavone). The IR spectrum of the methyl ether of **1** possessed bands at 1645 and 1675 cm^{-1}. Such spectral changes on methylation are reminiscent of the behaviour of flavanone and flavone systems [7] bearing hydroxyl groups at C-5. The UV spectrum of **1** showed absorption maxima at 255 sh, 265 and 320 nm. The shifts of the maxima in the presence of NaOMe, $AlCl_3$, $AlCl_3$–HCl, NaOAc and NaOAc–H_3BO_3 were also studied but did not prove to be very informative due to the superimposition of bands from the two flavonoid units. The structure was further elucidated with the help of ^{1}H, ^{13}C NMR and mass spectrometry.

Table 1. ^{13}C NMR spectral data of biflavonoid 1 (100 MHz, DMSO-d_6, TMS)

C	Flavanone	Flavone
4	196.04	181.56
7	168.44	163.47
5	163.73	160.46
9	162.78	155.20
4′	145.61	157.28
3′	145.58	114.39
1′	128.35	121.08
6′	119.18	128.32
5′	116.08	114.39
2′	113.26	128.10
10	103.14	102.21
6	96.14	98.56
8	95.21	100.49
2	80.88	161.57
3	48.29	101.46



1 R = H
2 R = Me



3

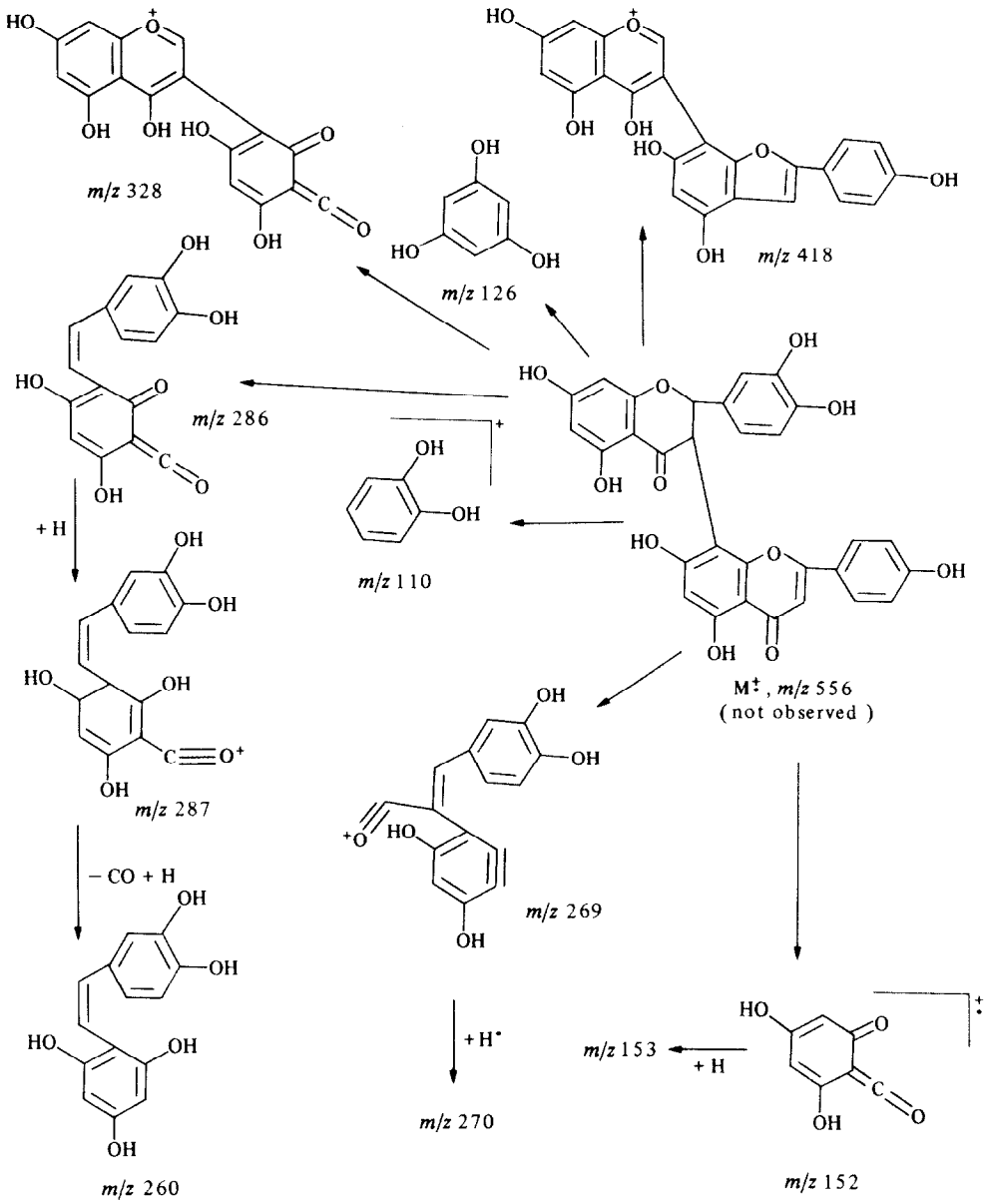
The decoupled ^{13}C NMR spectrum of **1** displayed 30 signals indicating that it was a biflavonoid. In the lowest field of the spectrum two signals were seen at δ 196.04 and 181.56 which confirmed the presence of a flavanone and a flavone unit in the molecule. The probability of a chalcone moiety was ruled out in view of the ^1H NMR spectral data. Ten signals in the region δ 145.58–166.44 were assignable to oxygenated aromatic carbons. The signals for the non-oxygenated aromatic carbons were observed in the range δ 95.21–128.65 as 16 signals. Two signals at δ 80.88 and 48.29 were attributed to C-2 and C-3, respectively, of the flavanone unit. The assignment of the ^{13}C NMR data was made by comparison with that of monoflavonoids [8] and is given in Table 1.

The ^1H NMR spectrum of **1** indicated the presence of seven hydroxyl groups by displaying seven singlets, each for one proton, in the lowest field of the spectrum at δ 9.27, 9.50, 9.83, 10.79, 11.18, 12.20 and 13.20. A pair of doublets ($J = 12$ Hz) at δ 5.64 and 4.83, each integrating for one proton was assignable to H-I-2 and H-I-3, respectively. The downfield shift of the C-3 proton relative to the chemical shift of the corresponding proton in the flavanone confirmed the involvement of the C-3 of the flavanone unit in the interflavonoidic linkage. A high field singlet in the aromatic region at δ 5.91 for two protons was attributed to the I-6 and I-8 protons. A downfield singlet at δ 6.17 for one proton was assignable to H-II-6 confirming the linkage of the flavone unit through C-8. A pair of doublets ($J = 8$ Hz), each integrating for two protons, at δ 6.32 (H-II-3′,5′) and 7.08 (H-II-2′,6′) was indicative of a p-hydroxylated B-ring. A singlet for one proton at δ 6.53 was assigned to the II-3 proton. A doublet ($J = 8$ Hz) for one proton at δ 6.84 (H-I-5′) and a multiplet for two protons centred at δ 7.37 (H-I-2′,6′) confirmed the presence of a 3, 4-dihydroxylated ring B structure.

It becomes obvious, from the above data, that compound **1** is a biflavonoid composed of a flavanone and a flavone unit. Also, ring A in both the units is derived from phloroglucinol and the C-3 of the flavanone unit is linked to C-8 of the flavone unit. There are, however, two probable structures **1** and **3** which could be assigned to this compound. Compound **3** is reported in the literature [9] and its mp, 300°, is much higher than that of **1**. The favourable structure is thus **1** which was finally confirmed with the help of mass spectral data.

Mass spectrometry which has been extremely helpful in the structural elucidation, especially for the interflavonoid linkage of biflavonoids provided evidence in favour of the 3/8-linkage as well as the nature of the B rings in **1**. The $[M]^+$, as is reported in the case of 3/8-hydroxylated biflavonoids, could not be observed [10] (scheme 1). The mass spectrum of methyl ether (**2**) also did not exhibit an $[M]^+$ peak, The major fragmentation path for **1** was the loss of phloroglucinol ring giving rise to the base peak at m/z 126. The most significant peak in the spectrum of **1** was at m/z 110 which could only be assigned to the dihydroxylated phenyl cation, The ion at m/z 418 may be explained in terms of the loss of ring B from the flavanone unit and CO from the flavone unit. The formation of an ion at m/z 260 can be explained by the RDA fragmentation of both the units accompanied by the loss of CO. The ring A fragments resulting from the RDA were observed at m/z 152 and 153. RDA by pathway II of the flavone unit and loss of ring A from the flavanone unit can explain the peaks at m/z 269 and 270. The peaks at m/z 286 and 287 can be explained in terms of RDA of both units. A peak at m/z 328 may arise from the RDA of the flavone unit and loss of B ring from the flavanone unit. The peaks at m/z 149 and 150 may be due to doubly charged ions arising from the fragment at m/z 328 by the loss of CO.

Thus, based on the above chemical and spectral considerations, compound **1** was characterised at I-5, II-5, I-7, II-7, I-3′, I-4′, II-4′-heptahydroxy-[I-3, II-8]-flavanonyl-flavone.



Scheme 1.

EXPERIMENTAL

Plant material. Plant material was collected from Zaria, Nigeria and a voucher specimen has been deposited at the herbarium of the Department of Botany, Aligarh Muslim University, Aligarh 202 001.

Extraction and isolation. Leaves of *G. nervosa* Mig. (G. andersoni Hook. f) (3 kg) were cut into pieces and exhaustively extracted with 95% EtOH (3 × 5 l). The extract was filtered, concd and defatted with petrol. The residue (700 g) was then extd with Et_2O. The Et_2O sol. portion was chromatographed over silica gel eluting successively with petrol, petrol–C_6H_6 mixts, C_6H_6, C_6H_6–EtOAc mixts and finally with EtOAc. Elution with C_6H_6–EtOAc (2:3) afforded impure **1** which was purified by prep. TLC using C_6H_6–EtOAc (1:1) and crystallized from C_6H_6–MeOH to give yellow crystals of I-5, II-5, I-7, II-7, I-3′, I-4′, II-4′-heptahydroxy-[I-3, II-8]-flavanonylflavone **1** (950 mg), mp 232–234°; IR ν_{max}^{KBr} cm^{-1}: 3300, 1745, 1640, 1610, 1510, 1460, 1380, 1260, 1160, 1030, 835; UV λ_{max} nm: (MeOH) 255, sh, 265, 320; (NaOMe) 258, 298, 362 sh, 385; ($AlCl_3$) 255, 285, 405; ($AlCl_3$–HCl) 255, 282, 335, 365; (NaOAc) 260, 300, 355 sh; (NaOAc–H_3BO_3) 245, 265, 305, 350; ^1H NMR (400 MHz, DMSO-d_6): δ 4.83 (1H, *d*, J = 12 Hz, H-I-3), 5.64 (1H, *d* = 12 Hz, H-I-2), 5.91 (2H, *s*, H-I-6,8), 6.17 (1H, *s*, H-II-6), 6.32 (2H, *d*, J = 8 Hz, H-II-3′,5′), 6.53 (1H, *s*, H-II-3), 6.84 (1H, *d*, J = 8 Hz, H-I-5′), 7.08 (2H, *d*, J = 8 Hz, H-II-2′,6′), 7.37 (2H, *m*, H-I-2′,6′), 9.27, 9.50, 9.83, 10.79, 11.18, 12.20, 13.20 (1H each, *s*, 7 × OH); MS *m/z*: 418, 328, 287, 286, 270, 260, 242, 153, 152, 126 (base peak) 110; ^{13}C NMR: see Table 1.

Heptamethyl ether of **1** (**2**). Compound **1** (100 mg) treated with Me_2SO_4–K_2CO_3 in Me_2CO for 24 hr and worked-up as usual yielded **2** (35 mg), mp 123–125°; IR ν_{max}^{KBr} cm^{-1}: 1675, 1645, 1600, 1515, 1400, 1375, 1260, 1155, 1110, 1040, 1020, 830; ^1H NMR (60 MHz, $CDCl_3$): δ 3.68, 3.74, 3.80, 3.84, 3.88, 3.90, 3.94 (3 H each, *s*, 7 × OMe), 4.91 (1 H, *d*, J = 13 Hz, H-I-3), 5.86 (1H, *d*, J = 13 Hz,

H-I-2). 6.20 (2 H, *s*, H-I-6,8), 6.30 (1 H, *s*, H-II-6), 6.50 (1 H, *s*, H-II-3), 6.60 (2 H, *d*, $J = 8$ Hz, H-II-3′, 5′), 6.98 (1H, *d*, $J = 8$ Hz, H-I-5′), 7.10–7.40 (4H, *m*, H-I-2′, 6′; H-II-2′, 6′).

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ALKALOIDS OF *THALICTRUM LANKESTERI*

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Key Word Index—*Thalictrum lankesteri*; Ranunculaceae; above-ground portion; bisbenzylisoquinoline alkaloid; hernandezine; protoberberine alkaloids.

Abstract—The isolation and identification of six alkaloids from an extract of the above-ground parts of *Thalictrum lankesteri* Standl. (Ranunculaceae) are described. The alkaloids isolated are the bisbenzylisoquinoline hernandezine, and the protoberberines berberine, palmatine, thalifendine, columbamine, and jatrorrhizine.

INTRODUCTION

Thalictrum lankesteri Standl. (Ranunculaceae), a perennial herb indigenous to Central America with a history of folkloric use as a medicinal plant, is a close botanical relative of other medicinally useful *Thalictrum* species [1]. Crude extracts of numerous species of *Thalictrum*, as well as pure alkaloids from these plants, have been used for centuries in the treatment of various ailments, some of which include cancer, jaundice, snakebite, rheumatism, and leprosy, as well as many other infections [2]. The genus *Thalictrum* is a well-known source of benzylisoquinoline-derived alkaloids, with over 220 of these bases (many possessing definable biological activities) having been isolated and identified [2]. It was decided to undertake a phytochemical investigation of the alkaloids of the above-ground parts of *Thalictrum lankesteri* because of the absence of reports in the literature concerning this species and because of the proclivity of the genus as a source of biologically active benzylisoquinoline-derived alkaloids. This paper reports the isolation of one non-quaternary and five quaternary alkaloids from an extract of the above-ground parts of *T. lankesteri*, a species indigenous to Costa Rica. These alkaloids include the bisbenzylisoquinoline hernandezine and the protoberberines berberine, palmatine, thalifendine, columbamine, and jatrorrhizine.

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RESULTS AND DISCUSSION

This is the first report of alkaloids in this species. Hernandezine has been shown to possess hypotensive, anti-inflammatory, hypothermic, and *in vitro* antimicrobial activities. In addition, the alkaloid was observed to produce an inhibition of conditioned avoidance reactions and motor-conditioned reflexes in rodents [2]. Berberine, palmatine, columbamine and jatrorrhizine have been shown to be inhibitors of *in vitro* microbial growth against a wide variety of organisms, some of which include *Mycobacterium smegmatis*, *Candida albicans*, *Saccharomyces carlsbergensis*, *Staphylococcus aureus*, and