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J. Nat. Prod., **1991**, 54 (3), 796-801• DOI:
10.1021/np50075a007 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 3, pp. 796-801, May-Jun 1991

TAXONOMIC STATUS OF *LANARIA LANATA* AND ISOLATION OF A NOVEL BIFLAVONE

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ABSTRACT.—Phytochemical examination of the EtOH extract of *Lanaria lanata* (= *L. plumosa*) showed the absence of phenalenone pigments that are the chemotaxonomic marker for the family Haemodoraceae. This finding, together with other embryological and palynological evidence, militates against inclusion of the monotypic South African *Lanaria* in that family. Investigation of the EtOAc-soluble materials resulted in the isolation of lanaroflavone [**1**], a novel biflavonoid, and the related amentoflavone [**3**].

Although both familial and tribal treatments of the monocotyledonous Haemodoraceae have been inconsistent, there seems to be general agreement that this small family of 14–17 genera and some 80 species comprises two tribes, the Haemodoreae and the Conostyleae (1–3). According to the latest treatment (2), the former comprises eight genera (*Barberetta, Dilatris, Haemodorum, Lachnanthes, Pyrrorhiza, Schiekia, Wachendorfia,* and *Xiphidium*) and the latter six (*Anigozanthos, Blancoa, Conostylis, Macropidia, Phlebocarya,* and *Tribonanthes*). The interrelationships among these genera have been clearly defined by taxonomy (1,2), palynology (3,4), chemistry (4), and embryology (5). Two additional genera, *Lophiola* and *Lanaria*, have been ascribed to the Haemodoraceae by various authorities; however, recent palynological (3,4) and chemical (4) work from our laboratory has indicated that *Lophiola* does not belong to the family; furthermore, the same pollen-ultramorphological study (3) has indicated that *Lanaria* should also be reassigned. *Lanaria* has, according to De Vos (5), an embryology which coincides best with that of the Tecophilaeaceae, and Dahlgren *et al.* (2) have suggested that the position of *Lanaria* may best be left open, although a position in the Tecophilaeaceae could be considered.

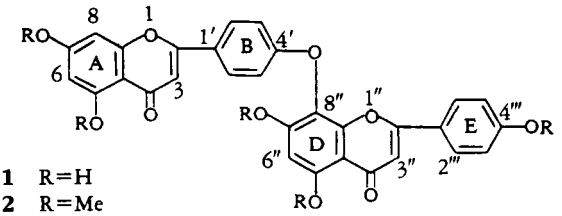
Because of the inconsistency between the different authorities on the position of this South African genus, we undertook an investigation of the secondary metabolites of *Lanaria* as a contribution to the natural classification of this species. All eight of the Haemodoraceous genera so far investigated have been found to contain pigments related to 9-phenyl phenalenone and/or 1*H*-naphtho[2,1,8-*mna*]xanthen-1-one, which are now considered to be chemotaxonomic markers for this family (6).

Lanaria lanata (L.) Dur. & Schinz (= *Lanaria plumosa* Ait.) grows in the Cape Province of South Africa; an exhaustive investigation of both the flowers and other parts of *L. lanata* has failed to give any indication of the expected pigments. This, together with the palynological (3) and embryological (5) evidence, militates strongly against the inclusion of this monotypic species in the Haemodoraceae. This present chemical study more firmly establishes the difference between *L. lanata* and other members of the Haemodoraceae than its affinity with other families. This is due to the paucity of detailed morphological, anatomical, palynological, embryological, and chemical data on the Tecophilaeaceae and some of the other families closely related to the Haemodoraceae (Hypoxidaceae, Liliaceae, Iridaceae, and Velloziaceae). Placement of *L. lanata* in any of these families would make that designation as tenuous as its present status.

RESULTS AND DISCUSSION

The present phytochemical study, while establishing the absence of phenalenones, has identified a novel biflavonoid, lanaroflavone, 4‴,5,5″,7,7″-pentahydroxy-4′,8″-

biflavonyl ether [**1**], which is an isomer of hinokiflavone [**6**]. The known biflavonoid amentoflavone [**3**] was also isolated.



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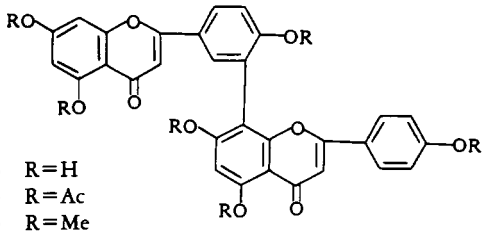
2 R=Me



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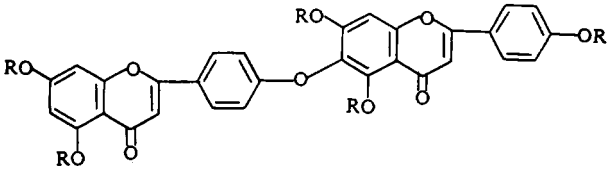
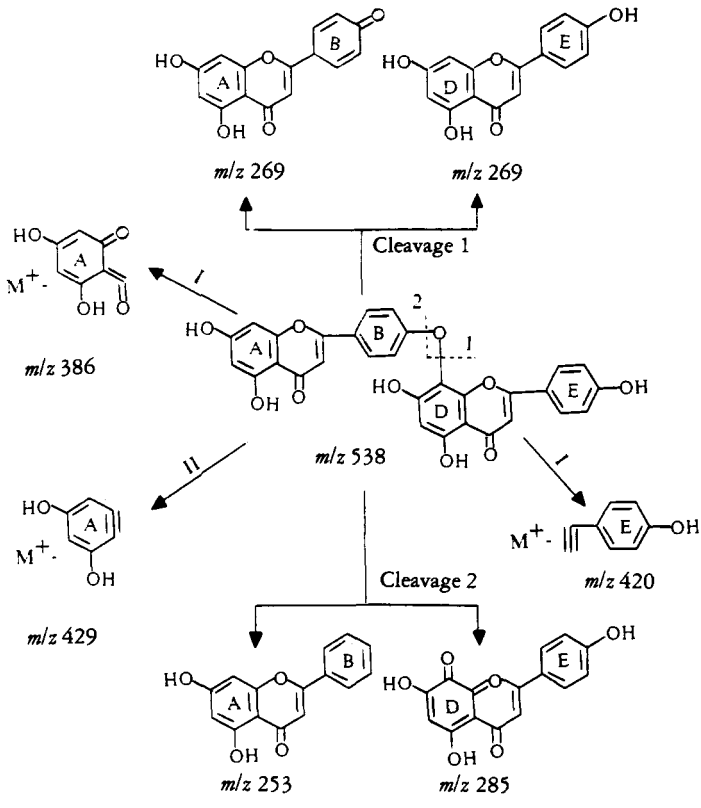
5 R=Me



6 R=H
7 R=Me

The EtOAc-soluble fraction of the aqueous EtOH extract of *L. lanata* was chromatographed on a Si gel column using $CHCl_3$ as eluent; the polarity was increased gradually with MeOH. MeOH (5%) eluted a major compound that, on rechromatography in the same system, gave 20 mg of a yellow compound, mp 294°, which gave a yellow color with NH_3 and a brown color with alcoholic $FeCl_3$. The hreims showed *m/z* 538.08998 $[M]^+$ (calcd 538.089 for $C_{30}H_{18}O_{10}$) and a fragmentation pattern typical of an ether-linked biflavonoid as shown in Scheme 1. Ions with *m/z* 253, 285, and 269 gave clear evidence of an ether-linked bisapigenin. Fragments with *m/z* 386 resulting from a retro-Diels-Alder reaction (pathway I) and *m/z* 429 (pathway II) exclude the possibility that the ether linkage is in the phloroglucinol part (ring A) of the molecule, and *m/z* 420 similarly shows that the ether linkage is not in ring E. The uv spectrum was similar to that of apigenin and underwent a bathochromic shift on addition of NaOMe or NaOAc or in the presence of $AlCl_3$ and with $AlCl_3$ + HCl, indicating the presence of hydroxyl groups at C-5 (or -3), -7, and -4‴ (7). Methylation of lanaroflavone [**1**] gave a pentamethyl ether **2**, $C_{35}H_{28}O_{10}$, $[M]^+$ 608. Acetylation gave a pentaacetate. The ^{1}H-nmr spectra of **1**, and **2**, and of the pentaacetate proved the presence of five OH groups and 13 aromatic protons in **1**, indicating it to be a biflavone with an ether linkage. Two of the five hydroxy resonances showed as downfield singlets at 12.90 and 12.78 ppm, indicating the two hydrogen-bonded OH groups at 5 and 5″. The presence of only two meta coupled doublets, each of one proton, in the upfield aromatic region at 6.56 (d, J = 1.9) and 6.28 (d, J = 1.9) ppm, which were assigned to H-8 and H-6, respectively, implies that either C-6 or C-8 in one of the phloroglucinol rings must be involved in the interflavonoid ether linkage. Eight of the thirteen aromatic protons appeared as two sets of AA′MM′ quartets readily assigned to the ortho coupled protons of rings B and E.



SCHEME 1. Mass spectral fragmentation of lanaroflavone [**1**]; I and II represent fragmentation pathways.

The three remaining aromatic sharp singlets (6.75, 6.70, and 6.50 ppm) were assigned to the protons at C-3, C-3″, and C-8″ (or C-6″), respectively.

The structure of **1** is therefore composed of two apigenin units with an ether linkage either between 4′-O-6″ (as in **6**) or 4′-O-8″. Lanaroflavone [**1**] has different physical (mp, tlc) and spectral properties from those of hinokiflavone [**6**], and the difference between the compounds was confirmed by studying the benzene shifts of the methoxy groups in the permethyl derivative of lanaroflavone. When C_6D_6 was added sequentially to the ^{1}H-nmr solution of **2** in $CDCl_3$, all the OMe resonances showed a continuous and significant upfield shift, implying that all five OMes in the permethylated derivative are adjacent to at least one unsubstituted carbon (8). This observation can be accommodated by structure **2** rather than by **7**, in which the OMe at C-5″ is flanked by two substituents. The ^{13}C nmr of **1** (Table 1) showed the presence of one signal at 94.00 and two signals at 99.17 and 98.92 ppm assigned to C-8, C-6, and C-6″, respectively (9,10). This implies that C-8″ is involved in the interflavonoid linkage. Comparison of the rest of the signals with those of **6** showed significant differences in the chemical shifts of the carbons of flavonoid II moiety as expected. Specifically, the signal for carbon 8″ in **1** is 26 ppm downfield from the corresponding resonance in **6** as would be expected if it were involved in the ether linkage (9), and carbons 5″, 7″, and 9″ (para and ortho to the ether linkage) are shielded with respect to those of the flavonoid I moiety by −4.0, −6.0, and −8.5 ppm, respectively.

A final proof of the proposed structure for lanaroflavone was the identity of the physical and spectral data of the permethylated derivative **2** with those of the known synthetic compound which had been characterized in the course of the structure determination of hinokiflavone [**6**] (11).

TABLE 1. ^{13}C nmr of the Biflavonoids Hinokiflavone [**6**], Lanaroflavone [**1**], and Amentoflavone [**3**].

Carbon	Compound		
	6	**1**	**3**
C-2	163.1	163.3	164.1
C-3	103.9	103.7	103.2
C-4	181.7	181.6	181.9
C-5	161.3	161.2	161.6
C-6	98.9	99.2	98.8
C-7	162.2	162.9	163.9
C-8	94.0	94.0	94.2
C-9	157.1	157.2	157.6
C-10	103.8	103.6	104.0
C-1′	124.1	124.6	120.3
C-2′	128.3	128.0	127.9
C-3′	115.3	115.5	121.7
C-4′	161.4	161.4	159.6
C-5′	115.3	115.5	116.4
C-6′	128.3	128.0	131.6
C-2″	164.2	164.2	164.3
C-3″	102.6	102.8	102.8
C-4″	182.1	181.7	182.2
C-5″	153.1	156.9	160.8
C-6″	124.7	98.9	99.1
C-7″	157.3	157.7	161.9
C-8″	94.6	120.7	104.1
C-9″	153.7	148.9	154.7
C-10″	104.1	104.0	104.0
C-1‴	121.1	121.1	121.4
C-2‴	128.5	128.4	128.3
C-3‴	116.0	115.8	116.0
C-4‴	160.6	160.9	161.1
C-5‴	116.0	115.8	116.0
C-6‴	128.5	128.4	128.3

The second compound **3** (1.5 g), yellow needles with mp 260°, gave an intense yellow color with NH_3 and brown with $FeCl_3$. The ir, ν max 3300 (hydroxyl groups), 1650 (conjugated-γ-pyrone), and uv absorption maxima in MeOH, were very similar to those of apigenin. The uv spectrum showed the characteristic bathochromic shift on addition of NaOMe, $AlCl_3$, $AlCl_3$ + HCl, and NaOAc. The fabms *m/z* 538 $[M]^+$, calculated for $C_{30}H_{18}O_{10}$. Acetylation afforded a hexaacetate **4**, mp 250°, $C_{42}H_{30}O_{16}$, $[M]^+$ 790, and methylation gave a hexamethyl ether **5**, mp 225°, $C_{36}H_{30}O_{10}$, $[M]^+$ 622. The ^1H-nmr spectra of the peracetate and the permethyl derivatives proved the presence of six OH groups and twelve aromatic protons in **3**, indicating that the compound was a biflavone with a C-C interflavonoid linkage. Four of the twelve aromatic protons appeared as a set of AA′MM′ doublets and were assigned to H-2‴, H-6‴, H-3‴, and H-5‴ in ring E. The signals at 8.12, 8.05, and 7.25 ppm were assigned to H-2′, H-6′, and H-5′ in ring B. The meta coupled doublets at 6.24 and 6.53 ppm were attributed to H-6 and H-8 for ring A. The three 1H singlets at 6.73, 6.66, and 6.46 correspond to the uncoupled protons H-3, H-3″, and H-6″ (or H-8″). These data are consistent with two flavone units linked through C-3′ of ring B to either C-8″ (i.e., amentoflavone) or C-6″ (i.e., robustaflavone) of the phloroglucinol ring D. Comparison

of the ^{13}C-nmr signals of the isolated compound with those reported for amentoflavone and robustaflavone (9) showed that the isolated compound to be amentoflavone. This finding was confirmed by studying the benzene shifts of the six OMe of the permethylated derivative **5**. All six showed a significant upfield shift, indicating that all of them have at least one ortho position unhindered, which is compatible with the structure of amentoflavone rather than that of robustaflavone. The identity of the isolated compound with amentoflavone was further demonstrated by comparative and co-tlc.

Biflavonoid compounds have been recorded in 32 families of flowering plants (12) but are rare in the monocots; recently, however, biflavonoids were identified in extracts of the monocotyledonous *Patersonia glabrata* (Iridaceae) (13), *Isophysis tasmanica* (Iridaceae), and *Xerophyta plicata* (Velloziaceae) (14), and interestingly, we have isolated a biflavonoid from *Lophiola aurea* (Tecophilaeaceae) (4). Furthermore, no flavonoids have been isolated from phenalenone-containing plants of the Haemodoraceae.

We feel that the chemical findings reported here, the absence of phenalenone and naphthoxanthenone pigments, and the presence of biflavonoids, in conjunction with the palynological evidence (3) and the embryological data (5), provide good evidence against the inclusion of *L. lanata* in the Haemodoraceae.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were determined in Me_2CO-d_6 or otherwise as mentioned on a Bruker 200 MHz spectrometer. The chemical shifts are given in ppm relative to TMS as internal reference. Hreims were recorded on a KRATOS MS 902 double focusing magnetic sector mass spectrometer. Fabms were recorded on a MAT 731 mass spectrometer. Ir spectra were obtained on a Beckman Acculab 3 in KBr. Uv spectra were obtained on a Varian CARY 2290 spectrophotometer in MeOH. Mp's were determined on a Koefler hot stage apparatus, and were uncorrected.

PLANT MATERIAL.—*L. lanata* was collected in 1986 in South Africa. The plants were dried and powdered. Voucher specimens are lodged in the herbarium at Kirstenboch Botanical Garden in Cape Town, South Africa.

EXTRACTION, FRACTIONATION, AND ISOLATION.—The powdered plant (1.5 kg) was extracted with 70% aqueous EtOH (3 liters × 3). The extracts were concentrated in vacuo to 300 ml. The concentrated aqueous EtOH extract was suspended in H_2O (400 ml) and partitioned sequentially with petroleum ether (40–60°), $CHCl_3$, EtOAc, and eventually with *n*-BuOH. Each fraction was concentrated to afford 50 g, 6 g, 13 g, and 35 g, respectively. The fractions were chromatographed using tlc to determine the presence or absence of phenalenones. No fraction contained any phenalenone pigments, but the $CHCl_3$, EtOAc, and *n*-BuOH fractions showed the presence of yellow pigments. The EtOAc fraction (13 g) was subjected to cc (200 g, Si gel, 2.5 × 50 cm glass column) using $CHCl_3$ and increasing proportions of MeOH. Fractions eluted with 5% MeOH contained one major compound. On evaporation, rechromatography on the same system, and crystallization from MeOH, lanaroflavone [**1**] (20 mg) was obtained.

LANAROFLAVONE [**1**].—Very fine yellow needles: 294–296°. Compound **1** gave a strong solvate-like jelly with MeOH and Me_2CO. Uv λ max 271 (log ε 4.4), 320–330 (log ε 4.37) (broad) nm, (NaOMe) 277, 390 nm, ($AlCl_3$) 280, 300, 340, 386 (broad) nm, ($AlCl_3$ + HCl) 282, 296, 339, 385 (broad) nm, (NaOAc) 280, 380 nm, (H_3BO_3) 270, 330 (broad) nm; ^{1}H nmr δ 8.09 (2H, d, J = 8.9 Hz, H-2′, -6′), 7.59 (2H, d, J = 8.8 Hz, H-2‴, -6‴), 7.26 (2H, d, J = 8.9 Hz, H-3′, -5′), 6.87 (2H, d, J = 8.8 Hz, H-3‴, -5‴), 6.75 (1H, s, H-3), 6.70 (1H, s, H-3″), 6.56 (1H, d, J = 1.9 Hz, H-6), 6.53 (1H, s, H-6″), 6.28 (1H, d, J = 1.9 Hz, H-8); ^{1}H nmr (DMSO-d_6) as with Me_2CO with two more singlets at 12.78 and 12.90 ppm exchangeable with D_2O (OH-5 and OH-5′); ^{13}C nmr see Table 1; hreims *m/z* (%) $[M]^+$ 538.08998 (5) ($C_{30}H_{18}O_{10}$ requires 538.089), 429 (7), 418 (9), 404 (7), 386 (5), 355 (15), 342 (20), 285 (15), 269 (100), 253 (20) (Figure 1); R_f 0.4 on Si gel with C_6H_6-pyridine-HCO_2H (20:5:1) (vs. 0.37 for hinokiflavone).

PERMETHYLLANAROFLAVONE [**2**].—Compound **1** (5 mg) was methylated with Me_2SO_4 in Me_2CO and Na_2CO_3 in the usual way to yield after chromatography in $CHCl_3$ over Si gel, compound **2** (5.5 mg) as an amorphous powder: mp 266° from MeOH [lit. (11) mp 268°]; ^{1}H-nmr ($CDCl_3$) δ 7.84 (2H, d, J = 9 Hz, H-2′, -6′), 7.44 (2H, d, J = 9. Hz, H-2‴, -6‴), 7.08 (2H, d, J = 9 Hz, H-3′, -5′), 6.83 (2H, d, J = 9 Hz, H-3‴, -5‴), 6.60 (1H, s, H-3), 6.58 (1H, s, H-3″), 6.54 (1H, s, H-6″), 6.54 (1H, overlapped, H-8), 6.37 (1H, d, J = 1.8 Hz, H-6), 4.06, 3.98, 3.95, 3.89, 3.79 (5 × 3H, s, 5 × OMe).

AMENTOFLAVONE [3].—Further elution of the initial column with 10% MeOH gave amentoflavone (1.5 g) as yellow needles from MeOH: mp 260° [lit. (15) mp 254–257°]; hrfabms *m/z* $[M+H]^+$ 539.0978 ($C_{30}H_{19}O_{10}$ requires 539.097); ir ν max 3300 (OH), 1645 (conjugated-γ-pyrone), 1600, 1575, 1500, 830 (*p*-substituted phenyl ring) cm^{-1}; uv λ max 268 (log ϵ 3.93), 334 (log ϵ 3.93) nm, (NaOMe) 274, 380 nm; ($AlCl_3$) 278, 300 (shoulder), 347, 385 nm; ($AlCl_3$ + HCl) 279, 300 (shoulder), 343, 385 nm; (NaOAc) 279, 388 nm; ^{1}H nmr δ 8.12 (1H, d, J = 2.1 Hz, H-2′), 8.05 (1H, dd, J = 2.1, 8.6 Hz, H-6′), 7.64 (2H, d, J = 8.7 Hz, H-2‴, -6‴), 7.25 (1H, d, J = 8.6 Hz, H-5′), 6.83 (2H, d, J = 8.7 Hz, H-3‴, -5‴), 6.73 (1H, s, H-3), 6.66 (1H, s, H-3″), 6.53 (1H, d, J = 1.4 Hz, H-8), 6.46 (1H, s, H-6″), 6.24 (1H, d, J = 1.4 Hz, H-6); ^{13}C nmr see Table 1.

PERACETYLAMENTOFLAVONE [4].—Amentoflavone (10 mg) was acetylated with pyridine and Ac_2O in the usual way to yield the peracetyl derivative (11 mg): mp 249° [lit. (15) mp 251–252°]; hreims $[M]^+$ 790.1533636 ($C_{42}H_{30}O_{16}$ requires 790.153); ir ν max 1760 (carbonyl of OAc), 1640 (conjugated-γ-pyrone), 1365, 1180 cm^{-1}.

PERMETHYLAMENTOFLAVONE [5].—Amentoflavone (10 mg) was methylated as for permethyllanaroflavone [2] and crystallized from MeOH to give 11 mg of the product **5**: mp 225° [lit. (14), mp 230–231.5°]; hreims *m/z* $[M]^+$ 622.183879 ($C_{36}H_{30}O_{10}$ requires 622.183); ^{1}H nmr ($CDCl_3$) δ 7.95 (1H, dd, J = 8.6, 2.2 Hz, H-6′), 7.87 (1H, d, J = 2.2 Hz, H-2′), 7.40 (2H, d, J = 9.0 Hz, H-2‴, -6‴), 7.15 (1H, d, J = 8.6 Hz, H-5′), 6.78 (2H, d, J = 9.0 Hz, H-3‴, -5‴), 6.66 (1H, s, H-3), 6.60 (1H, s, H-3″), 6.54 (1H, s, H-6″), 6.49 (1H, d, J = 2.1 Hz, H-8), 6.36 (1H, d, J = 2.1 Hz, H-6), 4.08, 3.95, 3.91, 3.85, 3.78, 3.77 (6 × 3H, s, 6 × OMe).

ACKNOWLEDGMENTS

We thank Dr. Catherine E. Costello (M.I.T.) for obtaining the fabms spectrum, Dr. Isao Kouno for providing a sample of hinokiflavone, Dr. J.B. Harborne for providing an authentic sample of amentoflavone, and Dr. Robert Highet (N.I.H.) for determining the ^{13}C-nmr spectrum of lanaroflavone.

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Received 29 August 1990