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Two novel flavans from *Cyperus conglomeratus*

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Separation of the extract of the underground tubers of *Cyperus conglomeratus* Rottb. (family Cyperaceae) afforded, in addition to known compounds, two novel flavans, which were identified, by one and two dimensional NMR, MS and IR spectra, as 5-hydroxy-7,3′,5′-trimethoxyflavan and 5,7-dihydroxy-3′,5′-dimethoxy-6-prenylflavan.

1. Introduction

Many *Cyperus* species are used extensively as medicinal plants [1–6] but only a few species of the family Cyperacea have been chemically investigated. Typical constituents of the family are flavonoids [7–10], quinones [11, 12], stilbenes [13, 14], sesquiterpenes [15–19], coumarins [20], triterpenes and sterols [21].

The species under investigation, *C. conglomeratus* Rottb., was only subjected to phytochemical screening which revealed the probable presence of flavonoids, coumarins and sterols [22]. In this article we report the isolation of eighteen natural products from this species, including two novel flavans exhibiting an unusual oxygenation pattern.

2. Investigations, results and discussion

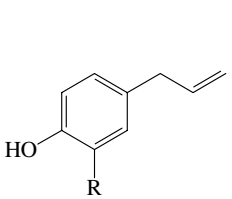
Chromatographic separation of the extract of the underground tubers of *C. conglomeratus* afforded five fatty acids, three terpenoids, two sterols, five aromatic shikimates and two new flavans. The fatty acids are *n*-caprylic [23], capric [24], myristic [25] methyl ester, palmitic [26] and linoleic [27] methyl ester. The methyl esters of myristic and linoleic were more likely formed due to methanolysis of fat material during extraction. The terpenoids are β-elemene [28], phytol and (4*R*,8*R*,12*R*)-4-hydroxy-4,8,12,16-tetramethylhepta-decanoic acid lactone [29]. The sterols are β-sitosterol and stigmasterol [30]. The shikimates are phenol [31], 1-phenylethanol [32], chavicol (**1**) [33], 3-ethoxy-4-hydroxyallylbenzene (**2**), the ethyl ester of allylcatechol [34] and isoferulic acid [35].

Compound **2** was identified from its MS which coincided well with the spectrum from the NIST library. This ethyl ether of allyl catechol is not found in the literature, so that we report its MS spectral data in the "Experimental" part.

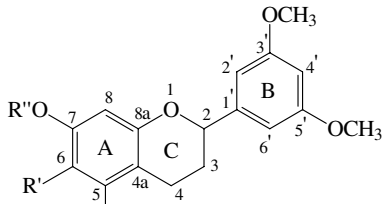
Known compounds were identified by comparing MS and/or ^{1}H NMR data with authentic spectra or literature data.

The ^{1}H NMR spectrum of compound **3** (Table 1) showed a double of doublet at δ 4.89 consistent with H-2 of flavans [36] (Table 1). The large coupling of 10.7 Hz pointed to 2,3-diaxial protons, in agreement with the preferable equatorial location of ring C. The proton spectrum exhibited also signals of two aromatic rings, one of them having two *meta*-related protons (δ 6.08, δ 6.13) and the other one exhibited a broad singlet of two protons at δ 6.92 and another one of one proton at δ 6.96, indicating the presence of a symmetrical 3′, 5′-disubstituted ring-B. The presence of three methoxyl group singlets at δ 3.75, 3.80 and 3.91 led to the conclusion of the molecular formula $C_{18}H_{20}O_5$ and that ring B is 3′, 5′-dimethoxy. The molecular ion peak M^+ at m/z 316 in the MS proved this. Moreover, the MS showed ion peaks due to $[B_2 + H]^+$ at m/z 167 and $A_2^{+\cdot}$ at m/z 150, confirming the proposed structure of ring-B and the presence of a hydroxyl group and a methoxyl group at ring-A.

The location of a methoxyl group at C-7 and a hydroxyl group at C-5 (in ring-A) was based on the NOE effects (Table 2) between the C-7-methoxyl group and H-6, H-8,



1; R = H
2; R = OEt



3; R' = H , R" = CH_3
4; R' = $CH_2CH{=}C(CH_3)_2$, R" = H

Table 1: ^{1}H NMR data of compounds 3 and 4 (δ-value, multiplicity (J Hz), 400 MHz, $CDCl_3$)

Compd.	**3**	**4**
H-2	4.89 dd (10.7, 2.0)	4.89 dd (10.5, 1.9)
H-3α	2.01 m	1.92 m
H-3β	2.16 m	2.15 m
H-4β	2.63 ddd (16.7, 11.6, 6.3)	2.62 ddd (16.7, 11.4, 6.3)
H-4α	2.78 ddd (16.7, 5.7, 2.4)	2.76 ddd (16.7, 5.7, 2.7)
H-6	6.08 d (2.3)	–
H-8	6.13 d (2.3)	6.03 s
H-2′, H-6′	6.92 br s	6.90 br s
H-4′	6.96 br s	6.95 br s
H-1p	–	3.35 d (7.0)
H-2p	–	5.27 t (7.0)
H-4p	–	1.73 br s
H-5p	–	1.70 br s
$OC\underline{H}_3$	3.75 s (at C-5′)	3.72 s (at C-5′)
	3.80 s (at C-3′)	3.86 s (at C-3′)
	3.91 s (at C-7)	–
OH	5.63 s	5.78 br s
	–	5.54 br s

Table 2: NOE effects, from NOESY spectrum of compounds 3 and 4

H-irradiated	affected H in **3**	affected H in **4**
2	3β > 3α, 4β, 2′, 6′	3β, 4β > 3α, 2′, 6′
3α	3β, 4β, 4α > 2	3β, 4β, 4α > 2, 2′, 6′
3β	3α, 4β, 4α, 2	3α, 4β, 4α, 2 > 2′, 6′
4β	4α, 3β, 2	4α, 3β, 2
4α	3α, 3β, 4β	3α, 3β, 4β
6	C-6-OCH_3, OH	–
C-7-OCH_3	6, 8	–
8	C-6-OCH_3, C-3′-OCH_3	C-3′-OCH_3, OH
2′, 6′	2,3α, 3β, OCH_3	2,3α, 3β, OCH_3
C-3′-OCH_3	8	8
C-5′-OCH_3	4′, 6′	4′, 6′
OH	6	1p, 8
1p	–	4p, 2p, OH, OH
2p	–	5p, 1p

as well as the effects between H-6 and the hydroxyl group. This was further confirmed by the long-range couplings (Table 3) between H-6 and C-8 (J^3), C-4a (J^3) as well as C-5 (J^2). Also the couplings between H-8 and both C-6 (J^3) and C-7 (J^2) supported the substitution pattern of ring-A. Thus **3** was identified as 5-hydroxy-7,3′, 5′-trimethoxyflavan. The ^{13}C-NMR spectrum (Table 4) was assigned using DEPT 90, DEPT 135, HXCOSY and HMBC spectra.

The ^{1}H and ^{13}C NMR spectra of **4** exhibited the signals of rings B and C similar to **3** (Tables 1 and 2). Ring A was represented by a singlet at δ 6.03 (assigned to H-8 based on the NOE effect with C-3′-OCH_3 group), signals of one prenyl (isopentenyl) group and two broad singlets of two OH groups. The presence of two cross peaks in the NOESY spectrum (Table 2) of the two OH signals with H-1p (of the prenyl group) indicated that the prenyl group is flanked by the two hydroxyl groups, in agreement with the proposed structure of **4** as 5,7-dihydroxy-3′, 5′-dimethoxy-6-prenylflavan. The ^{13}C NMR spectrum (Table 4) was assigned using DEPT 90, DEPT 135, HXCOSY and HMBC spectra. The MS fragmentation pattern (Scheme) confirmed the proposed structure.

These two flavonoids, **3** and **4**, are irregular with respect to the oxygenation pattern of the shikimate ring (ring B).

Table 3: Important correlations of compounds 3 and 4 in HMBC spectra

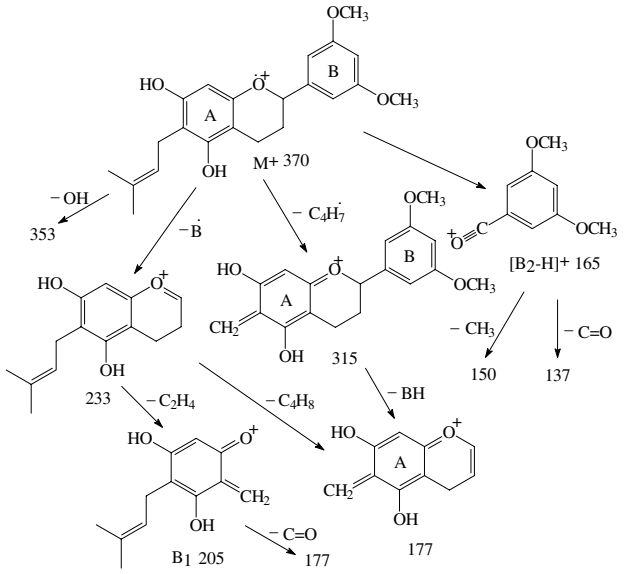
H-atom	correlated C in **3** (J)	correlated C in **4** (J)
H-2	1′ (J^2), 2′ (J^3)	1′ (J^2), 2′ (J^3), 6′ (J^3), 3 (J^2), 4 (J^3)
H-3α	2 (J^2)	2 (J^2)
H-3β	4a (J^3)	4a (J^3)
H-4β	3 (J^2), 4a (J^2), 8a (J^3)	3 (J^2), 2 (J^3), 4a (J^2), 5 (J^3), 8a (J^3)
H-4α	2 (J^3), 4a (J^2), 8a (J^3)	3 (J^2), 2 (J^3), 4a (J^2), 5 (J^3), 8a (J^3)
H-6	8 (J^3), 4a (J^3), 5 (J^2)	–
H-8	6 (J^3), 7 (J^2)	4a (J^3), 6 (J^3), 7 (J^2)
H-2′, H- 6′	2 (J^3), 4′ (J^3), 1′ (J^2), 3′, 5′ (J^2)	2 (J^3), 1′ (J^2), 3′ (J^2), 5′ (J^2)
H-4′	2′ (J^3), 3′, 5′ (J^2)	2′ (J^3), 3′, 5′ (J^2)
H-1p	–	3p (J^3), 2p (J^2), 6 (J^2), 7 (J^3)
H-2p	–	4p (J^3), 1p (J^2), 5p (J^3)
H-4p	–	5p (J^3), 2p (J^3), 3p (J^2)
H-5p	–	4p (J^3), 2p (J^3), 3p (J^2)
C-3′-OCH_3	–	3′ (J^3)
C-5′-OCH_3	–	5′ (J^3)

Table 4: ^{13}C NMR data of compounds 3 and 4 (δ-value, multiplicity[1], 100 MHz, $CDCl_3$)

Compd.	**3**	**4**
C-2	77.96 d	77.50 d
C-3	29.62 t	29.67 t
C-4	19.53 t	19.60 t
C-4a	103.35 s	103.11 s
C-5	159.34 s	156.39 s
C-6	91.40 d	106.76 s
C-7	158.58 s	153.63 s
C-8	93.39 d	91.62 d
C-8a	156.38 s	153.47 s
C-1′	133.64 s	134.25 s
C-2′	119.35 d	118.90[b] d
C-3′	146.58[a] s	146.49 s
C-4′	108.71 d	108.62 d
C-5′	145.38[a] s	145.01 s
C-6′	114.25 d	114.22[b] d
C-1p	–	22.00 t
C-2p	–	122.83 d
C-3p	–	133.58 s
C-4p	–	17.81 q
C-5p	–	25.79 q
OCH_3	55.34 q (at C-3′)	55.35 q (at C-3′)
	55.45 q (at C-5′)	55.88 q (at C-5′)
	55.94 q (at C-7)	–

[1] multiplicity was concluded from DEPT 90 and DEPT 135 experiments; a, b are exchangeable pairs

Scheme



MS fragmentation pattern of **4**

3. Experimental

3.1. General

GC/MS spectra were taken on a QP-7000 Shimadzu, with fused silica capillary column (30 m × 0.25 mm ID), film (5% phenyl, 95% methylsilicon) thickness 0.25 μm, and the output of an IBM computer with software Class 500 and NIST library for comparison; NMR spectra were recorded on Bruker FT-400 MHz; IR spectra were taken on a Nicolet Magenta 550 FT IR spectrometer.

3.2. Plant material

Cyperus conglomeratus Rottb. (family Cyperaceae) was collected on March 1998 from East Jeddah at the intersection of the ring road with Tahlia Street, K.S.A., and identified by Prof. Dr. Abdulaziz Faied, Botany Department, Faculty of Science, King Abdulaziz University. A voucher specimen is available at the first author among a private collection.

3.3. Processing of the plant material

The dried, ground underground tubers (800 g) of *Cyperus conglomeratus* were extracted by soaking at RT in petrol-Et_2O-MeOH (1 : 1 : 1) for 24 h. The crude extract (27.0 g) was defatted using cold MeOH.

3.4. Separation of the compounds

The defatted extract (19.5 g) was chromatographed on a silica gel CC into four fractions. Fraction I (1.2 g, eluted with petrol-Et_2O, 3 : 1) afforded by GC/MS β-elemene (2.3%, R_t 10.9 min), methyl myristate (9.2%, R_t 18.0 min) and methyl linoleate (18.5%, R_t 19.8 min). Fraction II (1.87 g, eluted with petrol-Et_2O, 1 : 1) gave by GC/MS phenol (6.0%, R_t 3.1 min), 1-phenylethanol (3.1%, R_t 4.3 min), chavicol (**1**) (3.6%, R_t 8.1 min), phytol (25.2%, R_t 20.0 min) and (4*R*,8*R*,12*R*)-4-hydroxy-4,8,12,16-tetramethylheptadecanoic acid lactone (10.9%, R_t 23.5 min). Fraction III was eluted by Et_2O as two successive subfractions IIIa and IIIb. Subfraction IIIa, when left overnight gave a precipitate (600 mg) which found by ^{1}H-NMR to be a mixture (1 : 1) of β-sitosterol and stigmasterol. The motherliquor of IIIa (240 mg dried material) was found by GC/MS to contain palmitic acid (14.3%, R_t 18.3 min). Subfraction IIIb (3.4 g) gave by GC/MS 1-phenyl-

ethanol (15.5%, R_t 4.3 min), octanoic acid (4.4%, R_t 6.1 min), decanoic acid (5.2%, R_t 10.1 min) and 3-ethoxy-4-hydroxyallylbenzene (**2**) (2.0%, R_t 17.2 min). Repeated TLC separation (silica gel, petrol-Et_2O, 1 : 1) trials of IIIb revealed the presence of a complicated mixture of flavans. Isoferulic acid was separated (20%, R_f 0.57). Fraction IV (5.2 g, eluted with Et_2O-MeOH, 9 : 1) afforded by TLC (silica gel, petrol-Et_2O, 1 : 3) cycloartenol (4%, R_f 0.83), the flavan **3** (10.5%, R_f 0.50) and the flavan **4** (60%, R_f 0.37).

3.5. 3-Ethoxy-4-hydroxyallylbenzene (2)

MS, m/z (rel. int.): 178 $[M]^+$ (100) (corresponding to $C_{11}H_{14}O_2$), 150 $[M-CO]^+$ (34.9), 124 $[150-C_2H_4]^+$ (25.6), 115 (11.6), 96 (14.0), 81 (18.6), 69 (26.7), 53 (27.9).

3.6. 5-Hydroxy-7,3′,5′-trimethoxyflavan (3)

Brownish-yellow gum; IR, $\upsilon_{max}^{CHCl_3}$, cm^{-1}: 3443.4 (OH), 2924.4 and 2849.2 (str. CH, CH_2, CH_3), 1616.5 and 1595.3 (C=C), 1514.3 (benzene ring), 1460.3, 1271.2, 1201.8, 1147.8, 1111.1, 1035.9, 816.0 and 756.2; ^{1}H-NMR: (Table 1); ^{13}C NMR: (Table 4); NOESY data: (Table 3); HMBC data: (Table 3); MS, m/z (rel. int.): 316 $[M]^+$ (100) (corresponding to $C_{18}H_{20}O_5$), 286 $[M-CH_2O]^+$ (27.4), 180 (4.4), 167 $[B_2 + H]^+$ (55.9), 150 $[A_2]^{+\cdot}$ (29.0), 137 $[167-CH_2O]^+$ (19.7), 135 (13.3), 77 (4.9).

3.7. 3′,5′-Dihydroxy-6,7-dimethoxy-4′-prenylflavan (4)

Brownish-red gum; IR, $\upsilon_{max}^{CHCl_3}$, cm^{-1}: 3439.5 (OH), 2926.4 and 2849.2 (str. CH, CH_2, CH_3), 1612.7 (C=C), 1516.2 (benzene ring), 1454.5, 1429.4, 1367.7, 1271.2, 1201.8, 1116.9, 1035.9, 904.7 and 758.1; ^{1}H NMR: (Table 1); ^{13}C NMR: (Table 4); NOESY data: (Table 2); HMBC data: (Table 3); MS, m/z (rel. int.): 370 $[M]^+$ (100) (corresponding to $C_{22}H_{26}O_5$), 353 $[M-OH]^+$ (3.9), 315 $[M-C_4H_7]^+$ (17.6), 233 (4.2), 221 (19.7), 205 (28.4), 177 $[205-CO]^+$ (15.2), 165 $[B_2\text{-}H]^+$ (14.0), 150 $[165-Me]^+$ (13.3), 137 [165−CO] (5.1), 107 (4.3), 77 (2.7).

References

1 Boulos, L.: Medicinal Plants of North Africa, p 80, Reference Publications Inc., Michigan (1983)
2 Dikshit, A.; Husain, A.: Fitoterapia **55**, 171 (1984)
3 Dini, A.; Ramunbo, E.; Saturion, P.; Scimone, A.; Stagno d'Alcontres, I.: Boll. Soc. Ital. Biol. Sper. **68**, 453 (1992); C.A. **119**, 45209s
4 Gilani, A. U.; Janbaz, K. H.: Gen. Pharmacol. **26**, 627 (1995); C. A. **122**, 230493e.
5 Gupta, S. K.; Sharma, R. C.; Aggarwal, O. P.; Arora, R. B.: J. Exp. Biol. **10**, 41 (1972); C.A. **77**, 43283b
6 Murase, T.; Hase, T.; Shibuya, J.; Nishizawa, Y.; Yada, Y.; Imokawa, G.: Jpn. Kokai Tokkyo Koho JP07,53,359,28 Feb 1995; JP Appl/ 140,654, 11Jun 1993; C.A. **122**, 299066k
7 Clifford, H. T.; Harborne, J. B.: Phytochemistry **8**, 123 (1969)
8 Harborne, J. B.: Phytochemistry **10**, 1569 (1971)
9 Harborne, J. B.; Williams, C. A.; Wilson, K. L.: Phytochemistry **24**, 751 (1985)
10 Williams, C. A.; Harborne, J. B.: Biochem. Syst. Ecol. **5**, 45 (1977)
11 Allan, R. D.; Wells, R. J.; Correll, R. L.; McLeod, J. K.: Phytochemistry **17**, 263 (1978)
12 Alves, A. C.; Moreira, M. M.; Paul, M. I.; Costa, M. A. C.: Phytochemistry **31**, 2825 (1992)
13 Nakajima, K.; Sato, S.; Sugama, H.; Mihashi, H.: Jpn. Kokai Tokkyo Koho JP04,159, 280, 02 Jun 1992; Appl.90/284, 307, 24 Oct 1990; C.A. **118**, 27445r
14 Powell, R. G.; Bajaj, R.; McLaughlin, J. L.: J. Nat. Prod. **50**, 293 (1987)
15 Garbarino, J. A.; Chamy, M. C.; Gambaro, V.: Phytochemistry **24**, 2726 (1985)
16 Hikino, H.; Aota, K.: Phytochemistry **15**, 1265 (1976)
17 Nerali, S. B.; Chakravarti, K. K.; Paknikar, S. K.: Indian J. Chem. **8**, 854 (1970)
18 Nyasse, B.; Tih, R. G.; Sondengam, B. L.; Martin, M. T.; Bodo, B.: Phytochemistry **27**, 179 (1988)
19 Uppal, S. K.; Chhabra, B. R.; Kalsi, P. S.: Phytochemistry **23**, 2367 (1984)
20 Dini, A.; Ramundo, E.; Saturino, P.; Scimone, A.; Stagno d'Alcontres, I.: Biochem. Syst. Ecol. **21**, 305 (1993).
21 Fedeli, E.; Cortesi, N.: Riv. Ital. Sostanze Grasse **47**, 252 (1970); C.A. **73**, 108455y
22 Rizk, A. M.: Fitoterapia **52**, 35 (1982)
23 Markley, K. S.: Fatty acids, Part 1, Interscience, N.Y., 2nd Ed. (1960)
24 Umemura, J.: J. Chem. Phys. **48**, 42 (1978)
25 Klein, W. J.: Recl. Trav. Chim. Pays-Bas **94**, 48 (1975)
26 Bailey, A. R.; Pittman, R. A.: J. Am. Oil. Chem. Soc. **48**, 775 (1971)
27 Ackman, R. G.; McLaughlan, J.; Scrimgeour, C. M.; Watson, A. P.: Chromatogr. Sci. **12**, 131 (1974)
28 Gri, R.; Marchesini, A.; Pagnoni, U. M.; Trave, R.: Phytochemistry **11**, 3363 (1972)
29 Abde-Mogib, M.; Basaif, S. A.; Ezmirly, S. T.: J. Saudi Chem. Soc. **2**, 141 (1998)
30 Abdel-Mogib, M.; Jakupovic, J.; Dawidar, A. M.; Metwally, M. A.; Abou-Elzahab, M.: Phytochemistry **29**, 2581 (1990)
31 Bancin, A. S.: Chem. Proc. Eng., 31 (1967)
32 Cram, D. J.; Kopecky, K. R.; Hauck, F.; Langemann, A.: J. Am. Chem. Soc. **81**, 5754 (1959)
33 Suga, T.; Iwata, N.; Asakawa, Y.: Bull. Chem. Soc. Jpn. **45**, 2058 (1972)
34 Ollis, W. D.; Somanathan, R.; Sutherl, I. O.: J. Chem. Soc., Chem. Commun., 494 (1974)
35 Bowden, B. F.; Cambie, R. C.; Parnell, J. C.: Aust. J. Chem. **28**, 91 (1975)
36 Ogawa, M.; Hisada, S.; Inagaki, I.: Yakugaku Zasshi **90**, 1081 (1970); C.A. **74**, 1107e

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