Pergamon

PII: S0031-9422(97)00001-0

Phytochemistry, Vol. 45, No. 4, pp. 825–829, 1997

0031-9422/97 $17.00+0.00

PRENYLATED FLAVANONES FROM *DERRIS RETICULATA*

CHULABHORN MAHIDOL,*† HUNSA PRAWAT* SOMSAK RUCHIRAWAT,*† KITTISAK LIHKITWITAYAWUID,‡§ LONG-ZE LIN and GEOFFREY A. CORDELL§¶

*Chulabhorn Research Institute, Bangkok, Thailand;† Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand; ‡Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

(*Received in revised form* 22 *November* 1996)

Key Word Index—*Derris reticulata*; Leguminosae; flavonoids; lupinifolin; 2‴,3‴-epoxylupinifolin; dereticulatin; NMR spectroscopy; cytotoxicity.

Abstract—Two new pyranoflavanones, 2‴,3‴-epoxylupinifolin and dereticulatin, together with the known flavonoid lupinifolin, were identified from the stems of *Derris reticulata*. The structures and NMR spectral data were assigned by the use of 2D NMR technology and chemical transformations. All of the isolates showed cytotoxic activity in the P-388 cell line.

INTRODUCTION

Derris reticulata Benth. (Leguminosae) is a medicinal plant of Thailand used for the relief of thirst and as an expectorant, and has not been previously studied chemically. Our recent studies on this plant led to the isolation of two new pyranoflavanones, 2‴,3‴-epoxylupinifolin (**2**) and dereticulatin (**3**), and a known flavonoid lupinifolin (**1**). In this report we present the isolation, structure determination, and biological evaluation of these compounds.

RESULTS AND DISCUSSION

Compound **1** was identified as lupinifolin by comparison of its mp, $[\alpha]_D^{20}$, UV, IR, ^{1}H and ^{13}C NMR, and mass spectrometry data with published values [1–3]. The ^{1}H and ^{13}C NMR spectroscopic assignments of **1**, as shown in Tables 1 and 2, were obtained through analysis of the COSY, NOESY, APT, HETCOR and selective INEPT [4, 5] spectra. It should be mentioned that the previous assignments of C-4a, C-5, C-6, C-7, C-8, C-8a and C-4′ [2] were revised, based on the 3-bond proton–carbon connectivities displayed in the selective INEPT experiments (Table 3). For example, a distinction between C-4a, C-6 and C-8 was made through the selective polarization transfer from H-3β (2.81 ppm), H-3″ (5.52 ppm) and H-2‴ (5.16 ppm) which enhanced the relevant resonances at 102.6, 102.79 and 108.73 ppm, respectively.

Isolate **2**, in HR mass spectrometry, exhibited $[M]^+$ *m/z* 422.1727, corresponding to the molecular formula $C_{25}H_{26}O_6$ (422.1729), and the UV absorptions at 226, 266, 274, 298, 311, 363 nm were reminiscent of a pyranoflavanone chromophore [1]. The ^{1}H NMR spectrum of **2** was similar to that of lupinifolin (**1**), except for the striking upfield position of the H-2‴ proton (δ 2.97), compared with that of **1** (δ 5.16). Although close resemblance was also observed between the ^{13}C NMR spectra of the two flavanones, the C-2‴ and C-3‴ carbons of **2** appeared to be oxygenated, resonating at δ 64.04 and 60.42, respectively, in contrast with those of **1**, which were olefinic. Comparison of the molecular formulae of **1** and **2** suggested an additional oxygen atom for **2**, which could be placed between C-2‴ and C-3‴, and in corroboration of this, the fragment ions at *m/z* 287 and 120 were demonstrated in the EIMS (Fig. 1). In addition, the linear pyranoflavanone structure of **2** was indicated by the 3-bond correlations between H-3″ and C-6, 5-OH and C-6, H-1‴ and C-7, and H-1‴ and C-8a in the HMBC [6, 7] spectrum (Table 4). Based on the above spectroscopic evidence, the structure of **2** was proposed to be 2‴,3‴-epoxylupinifolin. Careful examination of the ^{1}H and ^{13}C NMR spectra of **2**, however, revealed the composite nature of the isolate, as evident from the dual signals of the H-2, H-4‴, H-5‴ and 5-OH protons (Table 1) and the double signals of the C-2, C-3, C-4, C-7, C-2′(6′), C-3′(5′), C-4′, C-5″, C-6″ and C-1‴ carbons (Table 2). From these observations,

§ Present address: Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

¶ Author to whom correspondence should be addressed.

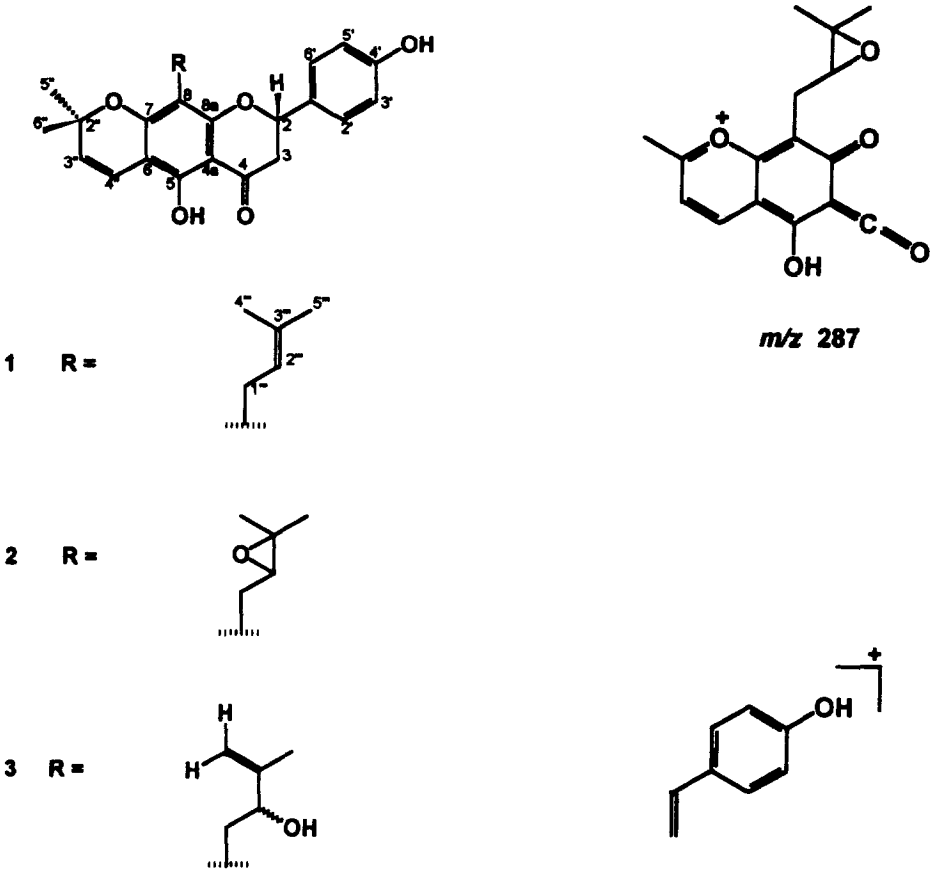

Fig. 1. Mass fragmentation of compound **2**.

4 = 5,4',2'''-triacetate of 3

it could be inferred that **2** was comprised of a pair of either conformational or configurational isomers. The former possibility was eliminated on the grounds that none of the dual proton signals merged when the spectrum was successively recorded at a high temperature (35°, 45°, 50° and 55°). On the other hand, oxidation of **1** with MMPP afforded a 1:1 mixture of 2'''-epimeric epoxide products which showed a ^{1}H NMR spectrum identical with that of **2**. Therefore, it was concluded that isolate **2** comprised the two 2'''-epimeric forms of the epoxide in 2''',3'''-epoxylupinifolin. Assignment of the ^{1}H and ^{13}C NMR resonances of **2** was accomplished through interpretation of the COSY, ROESY [8, 9], HMQC [10] and HMBC spectra, and the results are summarized in Tables 1 and 2.

Dereticulatin was assigned structure **3** through analysis of the NMR spectral properties of its triacetate derivative **4**. Apart from the three OAc resonances at δ 1.94, 2.32 and 2.43, the ^{1}H NMR spectrum of **4** also exhibited characteristic signals for H-2'(6') and H-3'(5') at δ 7.50 (*d*, J = 8.5 Hz) and 7.18 (*d*, J = 8.5 Hz), respectively [1], thereby placing one of the OAc groups at C-4'. Another OAc group was located at C-5, as indicated by the shielding effect on this carbon (δ 144.58) [2, 3]. Further examination of the ^{1}H NMR spectrum of **4** revealed the signals of H-3'' and H-4'' of the 2'',2''-dimethylpyran ring at δ 5.67 and 6.39. The diamagnetic shift of the H-4'' resonance required its placement *peri* to the 5-OAc group [11], thereby locating the pyran ring between C-6 and C-7 of ring A. As evidenced by the HMBC 3-bond correlation ($^3J_{CH}$ = 6 Hz) between the H_2-1''' protons and the C-8a carbon (which was assigned through selective INEPT irradiation of H-2, $^3J_{CH}$ = 3 Hz), the structure of **4** possessed a prenyl unit at C-8. In the COSY spectrum, the H_2-1''' methylene protons demonstrated vicinal coupling with the H-2''' methine proton, which was correlated to the oxygenated carbon at δ 76.13 in the HMQC spectrum; hence, the third OAc group was located at C-2'''. Finally, the presence of the 3''',4'''-vinyl functionality was deduced from HMQC correlation between the C-4''' (δ 112.20) and the H_2-4''' protons (δ 4.82 and 4.85, each *br s*), and this was confirmed by the 3-bond coupling between C-2''' and H_2-4''' in the HMBC spectrum. The ^{1}H and ^{13}C NMR resonances of **4** were assigned on the basis of its COSY, ROESY, HMQC and HMBC spectra, and are shown in Tables 1 and 2.

Table 1. ^{1}H NMR spectroscopic assignments of compounds **1**, **2** and **4**

Proton	1*	2†	4†
2	5.33 (*dd*, 12.6, 3.0)	5.25 (*dd*, 11.3, 3.0) 5.29 (*dd*, 11.3, 3.0)	5.40 (*dd*, 13.5, 3.0)
3α	3.06 (*dd*, 17.1, 12.6)	3.01 (*dd*, 17.2, 11.3)	2.97 (*dd*, 16.5, 13.5)
β	2.81 (*dd*, 17.1, 3.0)	2.77 (*dd*, 17.2, 3.0)	2.75 (*dd*, 16.5, 3.0)
2′(6′)	7.31 (*d*, 8.4)	7.20 (*d*, 8.5) 7.21 (*d*, 8.5)	7.50 (*d*, 8.5)
3′(5′)	6.89 (*d*, 8.4)	6.79 (*d*, 8.5)	7.18 (*d*, 8.5)
3″	5.52 (*d*, 10.1)	5.49 (*d*, 10.8)	5.67 (*d*, 10.0)
4″	6.64 (*d*, 10.1)	6.61 (*d*, 10.8)	6.39 (*d*, 10.0)
5″	1.45 (*s*)†	1.42 (*s*)‡	1.49 (*s*)‡
6″	1.46 (*s*)‡	1.44 (*s*)‡	1.50 (*s*)‡
1‴	3.22 (2H, *d*, 7.2)	2.68 (*m*) 2.94 (*m*)	2.88 (*dd*, 13.5, 8.0) 3.03 (*dd*, 13.5, 8.5)
2‴	5.16 (*dd*, 7.2, 7.2)	2.97 (*m*)	5.49 (*dd*, 8.5, 8.0)
4‴	1.66 (*s*)	1.27 (*s*)§, 1.28 (*s*)§	4.82 (*br s*) 4.85 (*br s*)
5‴	1.66 (*s*)	1.26 (*s*)§, 1.27 (*s*)§	1.73 (*s*)
5-OH	12.24 (*s*)	12.27, 12.28 (*s*)	
5-OCOCH$_3$			2.32 (*s*)§
4′-OCOCH$_3$			2.43 (*s*)§
2‴-OCOCH$_3$			1.94 (*s*)

* Recorded in $CDCl_3$ at 300 MHz; signal multiplicity and coupling constants (Hz) are in parentheses.
† Recorded in $CDCl_3$ at 500 MHz.
‡§ Interchangeable assignments within columns.

With regard to the biogenetic relationships of these flavanones, dereticulatin (**3**) could be considered to be derived from lupinifolin (**1**), with 2‴,3‴-epoxylupinifolin (**2**) as the intermediate. Oxidation of **1** would yield **2**, which would subsequently be transformed into **3** through enzymatic opening of the epoxide ring, coupled with the loss of a proton from one of the 3‴-methyl groups. Attempts to separate the mixture of 2‴-diastereomers of **2** and **3** were not successful.

In vitro bioassay evaluation [12, 13] of compounds **1**, **2** and the triacetate of **3** indicates that each of them inhibited the P-388 cell line at 0.4–0.5 μg ml^{-1}, but were inactive against the KB cell line.

EXPERIMENTAL

Mps uncorr.: on a Kofler hot-stage apparatus. Optical rotations measured with a Perkin-Elmer 241 polarimeter. UV spectra in MeOH on a Beckman DU-7 spectrometer. IR spectra in KBr on a MIDAC FT-IR interferometer. ^{1}H, ^{13}C, APT and COSY NMR spectra: recorded with either a Varian XL-300 or a General Electric GEΩ-500 spectrometer. NOESY and HETCOR spectra were obtained on a Varian XL-300 spectrometer. ROESY spectra were recorded with a GEΩ-500 instrument. Selective INEPT experiments were performed at 90.8 MHz using a Nicolet NMC-360 spectrometer. HMQC and HMBC spectra were obtained at 500.12/125.76 MHz using standard programs from the GE library. $^nJ_{C,H} = 6$ Hz was used for HMBC experiments.

Plant material. Dry stems of *Derris reticulata* were purchased from a local traditional drug store in Bangkok, Thailand, in December, 1990. Botanical identification was achieved through comparison with a specimen provided by Prof. Nijsiri Ruangrungsi. A herbarium voucher specimen is retained at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and isolation. Dry stems of *Derris reticulata* (2 kg) were powdered and extracted with CH_2Cl_2 at room temp. After filtration, the extract was evapd to give a brown solid (88 g). The brown solid was CC on Si gel eluted with a mixture of EtOAc–hexane (10:90, 15:85 and 20:80, respectively), successive frs were combined on the basis of their TLC behavior and evapd to give three frs. Fr. 1 (57 g), after crystallization from EtOAc–hexane, gave **1** as yellow needles (11 g, 0.00055%). Fr. 2 (150 mg) after rechromatography on Si gel with 15% EtOAc in hexane and crystallization from EtOAc–hexane, gave **2** (50 mg, 0.000025%), mp 84–85°C. Fr. 3 (280 mg), after processing in the same way as fr. 2, gave **3** (240 mg, 0.00012%), mp 162–168°; $[\alpha]_D^{20}$ +27.1° (Me_2CO, *c* 0.076; CD $[\theta]_{212}$ 0, $[\theta]_{216}$ +1820, $[\theta]_{222}$ +1.279, $[\theta]]_{233}$ +2900, $[\theta]_{256}$ 0, $[\theta]_{296}$ −5510, $[\theta]_{357}$ −330; MS *m/z* (%): 423 (M^+ +1, 2), 422 (5), 351 (72), 231 (100) and 120 (9).

Lupinifolin (**1**). Yellow crystals, mp 110°, $[\alpha]_D^{20}$ −9.9° ($CHCl_3$, *c* 1.1); CD $[\theta]_{211}$ 0, $[\theta]_{224}$ +18 007, $[\theta]_{281}$ −23 315, $[\theta]_{285}$ −22 560, $[\theta]_{298}$ −2957, $[\theta]_{317}$ 0, $[\theta]_{323}$ +3866, $[\theta]_{334}$ +2138, $[\theta]_{350}$ +558; UV λ_{max}^{MeOH} nm (log ε) 224 (4.38), 267 (4.68), 275 (4.72), 298 (4.17), 313 (4.15), 369 (3.55); IR ν_{max}^{KBr} cm^{-1} 3490–3216, 1644, 1520, 1462,

Table 2. ^{13}C NMR spectroscopic assignments of compounds **1**, **2** and **4**

C	1*	2†	4†
2	78.47	78.85, 78.96	78.71
3	42.97	43.23, 42.57	44.99
4	196.84	196.69, 196.73	189.10
4a	102.61	102.58	107.40
5	156.48	157.06	144.58
6	102.79	102.62, 102.70	109.25
7	160.13	160.06	157.88
8	108.73	104.23	
111.09			
8a	159.44	160.06	161.24
1′	130.60	129.56, 129.78	136.11
2′(6′)	127.66	127.52, 127.82	127.16
3′(5′)	115.53	115.48, 115.53	121.90
4′	156.09	156.70, 156.78	150.71
2″	78.20	78.53	78.31
3″	126.02	125.96	129.64
4″	115.53	115.42	115.15
5″	28.25‡	28.45,‡ 28.49‡	28.58
6″	28.33‡	28.33,‡ 28.39‡	28.58
1‴	21.42	21.71, 21.78	26.87
2‴	122.40	64.04	76.13
3‴	131.11	60.42	143.39
4‴	17.78	18.91	112.20
5‴	25.74	24.71	18.32
5-OCOCH$_3$			169.31
4′-OCOCH$_3$			169.31
2‴-OCOCH$_3$			169.98
5-OCOCH$_3$			21.07
4′-OCOCH$_3$			21.07
2‴-OCOCH$_3$			21.07

* Recorded in $CDCl_3$ at 75.6 MHz.
† Recorded in $CDCl_3$ at 125.76 MHz.
‡ Interchangeable assignments within columns.

1381, 1238, 1123, 889; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.) 406 $[M]^+$ (60), 392 (25), 391 (100), 363 (5), 351 (4), 271 (26), 243 (17), 215 (54), 120 (12).

2‴,3‴-*Epoxylupinifolin* (**2**). Yellow crystals, mp 84–85°, $[\alpha]_D^{20}$ +41.7° ($CHCl_3$, *c* 0.072); CD $[\theta]_{208}$ 0, $[\theta]_{225}$ +11 841, $[\theta]_{233}$ +11 758, $[\theta]_{259}$ 0, $[\theta]_{279}$ −12 270, $[\theta]_{283}$ −11 973, $[\theta]_{297}$ −16 290, $[\theta]_{319}$ 0, $[\theta]_{356}$ +2 736; UV λ_{max} (MeOH) nm (log ε) 226 (3.96), 266 (4.31), 274 (4.35), 298 (3.76), 311 (3.75), 363 (3.21); IR ν_{max}^{KBr} cm^{-1} 3490–3250, 1649, 1520, 1453, 1379, 1198, 1128; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS 422 $[M]^+$ (100), 408 (25), 407 (100), 351 (63), 350 (19), 335 (41), 287 (37), 231 (56), 215 (41), 120 (19). HR EIMS *m/z* found 422.1727 $[M]^+$, calcd $C_{25}H_{26}O_6$ 422.1729.

Table 3. Selective INEPT correlations of compound **1***

Proton	C
3α	(2),† (4), 1′
3β	(4), 4a
2′	2, 4′, 6′
3′	1′, (4′), 5′
3″	6, (2″)
4″	5, (6), 7, 2″
1‴	7, (8), 8a
2‴	8, (1‴), 4‴, 5‴
5-OH	4a, (5), 6

* $^3J_{CH}$ = 7 Hz for aromatic, olefinic and phenolic protons, and 6 Hz for methylene protons.
† Parentheses indicates 2-bond coupling.

Table 4. HMBC correlations of compounds **2** and **4**

Proton	2 (C)	4 (C)
3α	(2),*(4)	(2), (4), 1′
3β	(4)	
2′	2, 4′, 6′	2, 4′, 6′
3′	1′, (4′), 5′	1′, (4′), 5′
3″	(2″), 6	(2″, 6
4″	5, (6), 7, 2″	5, (6), 7, 2″,
5″	(2″), 3″	(2″), 3″
6″	(2″), 3″	(2″), 3″
1‴a	7, (8), 8a, (2‴), 3‴	7, 8, 8a, (2‴), 3‴
1‴b	7, (8), 8a, (2‴), 3‴	7, 8, 8a, (2‴), 3‴
2‴	(1‴)	4‴, (2‴-OCOCH$_3$)
4‴a,b	2‴, (3‴)	2‴, (3‴)
5‴	2‴, (3‴)	2‴, (3‴), 4‴
5-OH	4a, (5), 6	
5-OCOCH$_3$		(5-OCOCH$_3$)
4′-OCOCH$_3$		(4′-OCOCH$_3$)
2‴-OCOCH$_3$		(2‴-OCOCH$_3$)

* Parentheses indicates 2-bond coupling.

Epoxidation of lupinifolin (**1**). A soln of magnesium monoperoxyphthalate hexahydrate (MMPP, 775 mg, 1.3 mmol) in H_2O (4 ml) was added over 1 hr to a stirred soln of **1** (528 mg, 1.3 mmol) in $CHCl_3$ (3 ml) containing benzyltriethylammonium chloride (18 mg) which was kept at 50°. The mixt was stirred for a further 3 hr during which time the pH was maintained within the range 4.5–5.0 by the addition of aq. MeOH soln (5%). The aq. phase was extracted with $CHCl_3$ (3 × 25 ml), dried (Na_2SO_4), and evapd to afford a yellow solid (320 mg, 58.3%) which was recrystallized from EtOH–hexane to afford **2** as granules (200 mg), identical with the natural material.

Dereticulatin triacetates (**4**). Dereticulatin (**3**, 70 mg) was acetylated with Ac_2O–pyridene at room temp. with usual work-up to afford the triacetates **4**. Yellow crystals, mp 70°, CD $[\theta]_{225}$ +8294, $[\theta]_{260}$ 0, $[\theta]_{278}$ −6381, $[\theta]_{283}$ −5059, $[\theta]_{297}$ −7449, $[\theta]_{313}$ 0, $[\theta]_{322}$ +1866, $[\theta]_{360}$ +2501; UV λ_{max} nm (log ε) 222 (4.10), 260 (4.43), 294 (3.86), 341 (4.50); IR_{max}^{KBr} cm^{-1} 1769, 1740, 1605, 1468, 1372, 1204, 1125; EIMS *m/z* (rel. int.) 548 $[M]^+$ (2), 506 (10), 446 (8), 431 (6), 394 (22), 393 (100), 269 (9), 232 (5), 231 (36), 215 (8), 189 (7), 120 (10). HR EIMS *m/z* found 548.2051 $[M]^+$, calcd $C_{31}H_{32}O_9$ 548.2046.

Acknowledgements—This work was supported, in part, by a grant from the Thai Government and the Petroleum Authority of Thailand to the Thai research

groups. We thank the Research Resources Center of the University of Illinois at Chicago for the provision of the NMR facilities, and Dr G. Doss for the initial implementation of the selective INEPT NMR technique at UIC.

REFERENCES

1. Smalberger, T. M., Vleggaar, R. and Weber, J. C., *Tetrahedron*, 1974, **30**, 3927.
2. Van Zyl, J. J., Rall, G. J. H. and Roux, D. G., *Journal of Chemical Research* (*S*), 1979, 97.
3. Van Zyl, J. J., Rall, G. J. H. and Roux, D. G., *Journal of Chemical Research* (*M*), 1979, 1301.
4. Cordell, G. A., *Phytochemical Analysis*, 1991, **2**, 49.
5. Cordell, G. A. and Kinghorn, A. D., *Tetrahedron*, 1991, **47**, 3521.
6. Summers, M. F., Marzilli, L. G. and Bax, A., *Journal of the American Chemical Society*, 1986, **108**, 4285.
7. Bax, A., Aszalosm A., Dinya, Z. and Sudo, K., *Journal of the American Chemical Society*, 1986, **108**, 8056.
8. Bothner-By, A. A., Stephens, R. L., Lee, L., Waren, C. D. and Jeanloz, R. W., *Journal of the American Chemical Society*, 1984, **106**, 811.
9. Griesinger, G. and Ernst, R. R., *Journal of Magnetic Resonance*, 1987, **75**, 261.
10. Bax, A. and Subramanian, S., *Journal of Magnetic Resonance*, 1986, **67**, 565.
11. Mahmoud, E. N. and Waterman, P. G., *Journal of Natural Products*, 1985, **48**, 648.