Pergamon 0031-9422(95)00316-9

Phytochemistry, Vol. 40, No. 2, pp. 577–581, 1995

0031-9422/95 $9.50 + 0.00

FLAVONOL SINAPOYL GLYCOSIDES FROM LEAVES OF *THEVETIA PERUVIANA**

FUMIKO ABE, YUKIKO IWASE, TATSUO YAMAUCHI,† SHOJI YAHARA‡ and TOSHIHIRO NOHARA‡

Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-80, Japan; ‡Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862, Japan

(*Received in revised form* 7 *February* 1995)

Key Word Index—*Thevetia peruviana*; Apocynaceae; kaempferol; quercetin; flavonol sinapoyl bioside; kaempferol bis-sinapoyl trioside.

Abstract—From the leaves of *Thevetia peruviana* four new flavonol glycosides, kaempferol 3-glucosyl(1 → 4) [6‴-sinapoylglucosyl] (1 → 2) galactoside and 3-[2‴″-sinapoylglucosyl](1 → 4)[6‴-sinapoylglucosyl](1 → 2)galactoside and kaempferol and quercetin 3-[6‴-sinapoylglucosyl](1 → 2)galactoside were isolated, together with the known compounds, kaempferol and quercetin 3-glucosyl(1 → 2)galactoside. The glycosides were characterized from FAB mass, ^{1}H and ^{13}C NMR and UV spectral data.

INTRODUCTION

During an investigation of the constituents of *Thevetia peruviana* (Pers.) K. Schum., we have described cardenolides [1–3] and iridoids [4, 5] from the leaves and roots. The present paper deals with the isolation and characterization of kaempferol and quercetin glycosides with sinapic acid residues attached to the sugar moiety from leaf tissue of the same plant.

RESULTS AND DISCUSSION

Six flavonol glycosides (**1**–**6**) were obtained from a methanolic leaf extract of *T. peruviana* by column chromatography and preparative HPLC. Kaempferol was identified as the aglycone in **1**–**4** from the characteristic signals due to H-6, H-8, H-2′, H-3′, H-5′ and H-6′ along with the corresponding ^{13}C signals as observed in the spectra of kaempferol glycosides.

In glycoside **1**, $C_{27}H_{30}O_{16}$, all ^{1}H and ^{13}C signals were assigned based on ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra. While the ^{13}C signals due to D-glucose were observed at the normal chemical shifts, C-2 of galactose showed a lower field shift. The linkage of D-glucose to D-galactose was confirmed by the difference in NOE measurement, where the H-2 signal of galactose responded when the anomeric proton signal of D-glucose was irradiated. Compound **1** was identified as the known glycoside kaempferol 3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranoside [6].

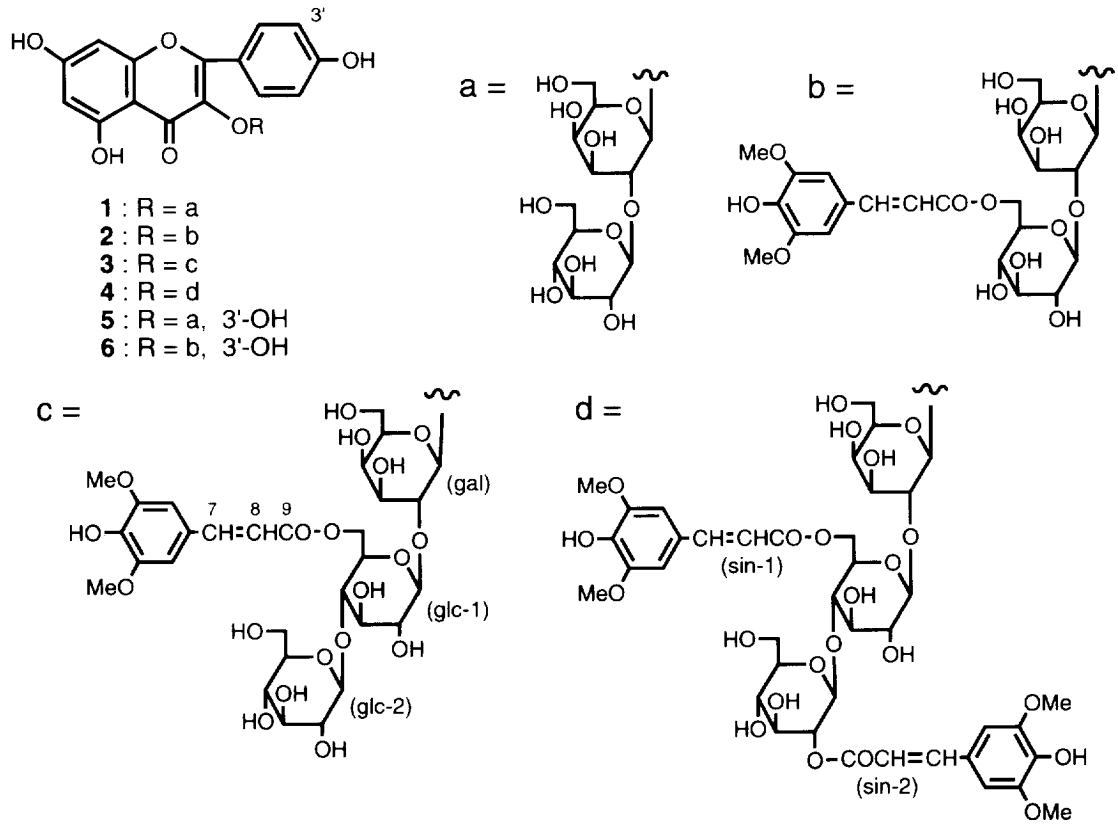
In addition to the ^{1}H and ^{13}C NMR signals observed in **1**, glycoside **2** showed the ^{1}H signals due to two methoxyl groups, two phenyl protons at δ6.83 (2H, *s*), and *trans* olefinic protons at δ6.47, 7.78 (each *d*, J = 16 Hz). as well as the ^{13}C signals for one carbonyl (δ167.4), six phenyl (δ106.6(×2), 125.0, 140.5, 149.1(×2)), two olefinic carbons (δ115.1, 145.8) and two methoxyl groups. The FAB mass spectrum afforded a $[M + Na]^+$ peak at m/z 839.2012, suggesting the molecular formula to be $C_{38}H_{40}O_{20}$, consistent with a structure with one more sinapoyl residue than in **1**. The location of the sinapic acid was determined to be at the C-6 hydroxyl group of the terminal glucose unit, based on acylation shifts of its H-6a and b.

The FAB mass spectrum of **3** afforded a $[M + Na]^+$ peak at m/z 1001.2546 ($C_{44}H_{50}O_{25}Na$), suggesting the structure had one more hexose than **2**. In the ^{1}H NMR spectrum, one additional anomeric proton signal was observed at δ5.04, besides two in **2** at δ6.25 and 5.37, all with 8 Hz coupling constants. The ^{13}C signals showed that **3** was composed of **2** plus an additional hexose, which was assignable as glucose. The linkage to **2** was determined to be C-4 of the glucose, based on the glycosylation shift of C-4_{glc-1}, and the cross peak between H-1_{glc-2}/C-4_{glc-1} was observed as well as that of H-1_{glc-1}/C-2_{gal} in the HMBC spectrum. Therefore, compound **3** was characterized as kaempferol 3-*O*-β-D-glucopyranosyl(1 → 4) [6‴-*O*-sinapoyl-β-D-glucopyranosyl] (1 → 2)-β-D-galactopyranoside.

Glycoside **4** showed a $[M + Na]^+$ peak at m/z 1207.3119 ($C_{55}H_{60}O_{29}Na$), suggesting a similar structure to **3** with one additional sinapoyl residue. In fact, two sets of signals due to phenyl, *trans* olefinic, and methoxyl groups were observed besides three anomeric proton

*Part 6 in the series '*Thevetia*'. For Part 5, see ref. [5].

†Author to whom correspondence should be addressed.



signals at δ6.28, 5.36 and 5.22. The signals due to the sugar moiety were assigned based on the ^{1}H–^{1}H COSY and HOHAHA measurements. In the ^{13}C NMR spectrum, all signals due to **3** and one additional sinapoyl residue were also assignable. The second sinapic acid was located to the C-2 hydroxyl of the external glucose unit based on the cross peak between H-2$_{glc\text{-}2}$ and the carbonyl carbon (δ166.8) of the second sinapic acid in the HMBC spectrum.

The aglycones of **5** and **6** were both characterized as quercetin based on the ABX coupling pattern due to ring B. The sugar moiety of **5** was identified as *O*-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranose and located at the C-3 hydroxyl by comparison of the ^{1}H and ^{13}C NMR spectra with those of **1**. A $[M + Na]^+$ peak at *m/z* 649.1379 ($C_{27}H_{30}O_{17}Na$), one oxygen larger than **1**, was coincident with the proposed structure. Similarly, **6** ($C_{38}H_{40}O_{21}$) was determined to be quercetin 3-*O*-[6‴-*O*-sinapoyl-β-D-glucopyranosyl](1 → 2)-β-D-galactopyranoside by comparison of the NMR spectra with those of **5** and **2**.

Only two kaempferol 3,7-bisglycosides having sinapic acid residues attached to the sugar moiety have been reported previously [7, 8]. Four additional sinapoyl flavonol glycosides, **2**–**4** and **6**, are recorded here for the first time from nature.

EXPERIMENTAL

General. ^{1}H NMR: 400 or 500 MHz; and ^{13}C NMR: 100 or 125 MHz in pyridine-d_5 and TMS as int. standard. UV spectra were measured in MeOH. For TLC and silica gel CC, the following solvent systems were used, 1: $CHCl_3$–MeOH–H_2O (7:3:1, bottom layer); 2: EtOAc–MeOH–H_2O (8:2:1–7:2:1, upper layer). Spray reagents for TLC: (1) 10% H_2SO_4; (2) 10% $FeCl_3$.

Plant material. Isolation of flavonoids was carried out with the same plant material of *T. peruviana* (Pers.) K. Schum. used for the investigations on cardenolides [3] and iridoids [4].

Extraction and isolation of flavonoids. The leaves of *T. peruviana* (1.5 kg), stored at −20° immediately after collection, was percolated with cold MeOH. The MeOH percolate was dissolved in 50% MeOH and defatted with benzene (benzene ext. 11.2 g). The MeOH–H_2O layer was concentrated *in vacuo* and passed through a polystyrene column (MCI-gel CHP-20P, Mitsubishi Chem. Ind.). The eluate with 75% MeOH containing cardiac glycosides was re-chromatographed on a ODS column (Fuji Gel Ind.) with 20%–40% MeOH. The fraction showing a positive reaction with $FeCl_3$ reagent was then chromatographed on a silica gel column with solvents 2 and 1 repeatedly and finally subjected to prep. HPLC (ODS, 25% CH_3CN). Six flavonol glycosides were isolated: **1** (31 mg), **2** (11 mg), **3** (13 mg), **4** (12 mg), **5** (7 mg), **6** (3 mg).

Kaempferol 3-O-β*-*D*-glucopyranosyl*(1 → 2)-β*-*D*-galactopyranoside* (**1**). A solid, $[\alpha]_D^{26}$ − 28.1° (MeOH; *c* 0.41), FABMS *m/z*: 633.1432 (Calcd for $C_{27}H_{30}O_{16}Na$: 633.1431). UV λ_{max} nm (log ε): 266 (4.26), 299 (4.05), 324 (4.11), 338 (4.15), 350 (4.16). ^{1}H and ^{13}C NMR: see Tables 1 and 2.

Table 1. ^{1}H NMR spectral data for flavonoids **1**–**5** and **6** [δ (ppm) in pyridine-d_5 (400 MHz)]

H	1	2*	3*	4†	5*	6
6	6.67 *d* (2)	6.62 *d* (2)	6.61 *d* (2)	6.59 *d* (2)	6.65 *d* (2)	6.60 *d* (2)
8	6.64 *d* (2)	6.58 *d* (2)	6.56 *d* (2)	6.54 *d* (2)	6.53 *d* (2)	6.47 *d* (2)
2′	8.50 *d* (9)	8.53 *d* (9)	8.50 *d* (9)	8.46 *d* (9)	8.35 *d* (2)	8.28 *d* (2)
3′	7.27 *d* (9)	7.28 *d* (9)	7.28 *d* (9)	7.24 *d* (9)		
5′	7.27 *d* (9)	7.28 *d* (9)	7.28 *d* (9)	7.24 *d* (9)	7.35 *d* (8)	7.34 *d* (8)
6′	8.50 *d* (9)	8.53 *d* (9)	8.50 *d* (9)	8.46 *d* (9)	8.09 *dd* (8, 2)	8.27 *dd* (8, 2)
5-OH	13.42 *br s*	13.35 *br s*	13.36 *br s*	13.36 *br s*	13.52 *br s*	13.44 *br s*
gal						
1	6.57 *d* (8)	6.34 *d* (8)	6.25 *d* (8)	6.28 *d* (8)	6.48 *d* (8)	6.47 *d* (8)
2	4.88 *dd* (8, 9)	4.89 *t* (8)	4.83 *dd* (8, 9)	4.82 *dd* (8, 9)	4.88 *t* (8)	4.92 *dd* (8, 9)
3	4.34 *dd* (9, 3)	4.29 *dd* (8, 3)	4.23 *dd* (9, 3)	4.25 *dd* (9, 3)	4.35 *dd* (8, 3)	4.13–4.36
4	4.56 *br d* (3)	4.53 *br d* (3)	4.52 *br d* (3)	4.51 *br d* (3)	4.54 *br d* (3)	4.54 *br d* (3)
5	4.08 *br t* (6)	4.01 *br t* (6)	3.98 *br t* (6)	3.98 *br t* (6)	4.06 *br t* (6)	4.04 *br t* (6)
6	4.16–4.34	4.13–4.29	4.00–4.29	4.10–4.29	4.17–4.36	4.13–4.36
glc-1						
1	5.43 *d* (7)	5.43 *d* (7)	5.37 *d* (7)	5.36 *d* (7)	5.43 *d* (7)	5.49 *d* (7)
2	4.19 *dd* (7, 8)	4.15 *dd* (7, 8)	4.00–4.29	4.14–4.20	4.16 *dd* (7, 8)	4.13–4.36
3	4.16–4.34	4.20 *t* (8)	4.00–4.29	4.14–4.20	4.17–4.36	4.13–4.36
4	4.16–4.34	4.13–4.29	4.00–4.29	4.14–4.20	4.17–4.36	4.13–4.36
5	3.83 *m*	4.02 *m*	4.00–4.29	3.90 *m*	3.94 *m*	4.13–4.36
6a	4.40 *dd* (12, 2)	4.90 *dd* (12, 1)	5.10 *br d* (2)	4.75 *br d* (12)	4.47 *dd* (12, 2)	4.96 *br d* (12)
6b	4.16–4.34	5.01 *dd* (12, 5)	5.10 *br d* (2)	4.85 *dd* (12, 4)	4.17–4.36	5.07 *dd* (12, 4)
glc-2						
1			5.04 *d* (8)	5.22 *d* (8)		
2			4.00–4.29	5.76 *dd* (8, 9)		
3			4.00–4.29	4.33 *t* (9)		
4			4.00–4.29	4.16 *t* (9)		
5			3.97 *m*	4.12 *m*		
6a			4.59 *dd* (11, 2)	4.61 *dd* (12, 2)		
6b			4.26 *dd* (11, 4)	4.23 *dd* (12, 5)		
sin-1				(sin-2)		
2, 6		6.83 *s*	6.83 *s*	6.81 (7.10) *s*		6.83 *s*
7		7.78 *d* (16)	7.76 *d* (16)	7.59 (8.02) *d* (16)		7.77 *d* (16)
8		6.47 *d* (16)	6.52 *d* (16)	6.47 (6.83) *d* (16)		6.46 *d* (16)
–OMe		3.79 *s*	3.79 *s*	3.79 (3.81) *s*		3.79 *s*

Coupling constants (J in Hz) given in parentheses.
*Signal assignments were based on ^{1}H–^{1}H COSY spectra.
†Signal assignments were based on ^{1}H–^{1}H COSY spectrum and HOHAHA measurement.

Table 2. ^{13}C NMR spectral data for flavonoids **1**–**5** and **6** [δ ppm in pyridine-d_5 (100 MHz)]

C	1	2	3	4	5	6
2	156.6	156.8	157.3	156.8	156.9	156.7
3	134.4	134.6	134.7	134.5	134.7	134.7
4	178.9	179.0	179.0	178.9	179.0	178.9
5	162.9	162.8	162.8	162.8	162.9	162.8
6	99.7	99.6	99.6	99.5	99.6	99.5
7	165.6	165.6	165.5	165.5	165.5	165.4
8	94.4	94.3	94.3	94.3	94.3	94.2
9	157.4	157.3	156.8	157.3	157.4	157.3
10	105.3	105.2	105.2	105.2	105.2	105.2
1′	122.1	122.0	122.0	121.9	122.4	122.7
2′	132.0	132.0	132.0	131.9	116.3	116.3
3′	116.2	116.2	116.2	116.1	146.6	146.7
4′	161.5	161.5	161.5	161.5	150.4	150,5

Cont. overleaf

Table 2. (*Continued*)

C	1	2	3	4	5	6
5′	116.2	116.2	116.2	116.1	117.9	117.4
6′	132.0	132.0	132.0	131.9	122.8	122.9
gal						
1	100.4	100.7	100.7	100.6	100.7	100.5
2	82.5	82.3	82.8	82.5	82.9	82.6
3	75.3	75.1	75.0	75.1	75.3	75.1
4	69.5	69.6	69.5	69.5	69.5	69.0
5	77.4	77.1	77.1	77.1	77.4	77.3
6	61.6	61.5	61.5	61.5	61.5	61.5
glc-1						
1	106.1	105.2	105.6	105.5	106.3	106.0
2	76.0	75.9	75.4*	75.3*	76.0	76.0
3	78.5*	78.1	76.4*	76.4*	78.4*	78.1
4	71.4	71.1	81.3	82.3	71.2	71.0
5	78.4*	75.7	73.9	73.3	78.3*	75.7
6	62.5	64.2	63.9	63.8	62.2	64.2
glc-2						
1			105.2	102.9		
2			74.7	74.9		
3			78.3	76.4		
4			71.8	72.0		
5			78.6	78.7		
6			62.7	62.5		
sin-1				(sin-2)		
1		125.0	125.1	125.3 (125.0)		125.1
2		106.6	106.6	106.6 (107.0)		106.6
3		149.1	149.0	149.0 (149.0)		149.0
4		140.5	140.4	140.4 (140.5)		140.4
5		149.1	149.0	149.0 (149.0)		149.0
6		106.6	106.6	106.6 (107.0)		106.6
7		145.8	145.8	145.8 (146.5)		145.7
8		115.1	115.2	114.8 (115.5)		115.2
9		167.4	167.3	167.3 (166.8)		167.4
–OMe		56.3	56.3	56.2 (56.2)		56.3

*Interchangeable within the same column.

Table 3. Cross-peaks in HMBC spectra of **3** and **4**

	3			**4**	
	C			C	
H	3-bond	2-bond	H	3-bond	2-bond
6	8, 10		5-OH	6, 10	
8	6, 10	9	6	8, 10	
2′, 6′	4′		8	6, 10	9
3′, 5′	1′		2′, 6′	4′, 2	
7 (sin-1)	2, 9 (sin-1)		3′, 5′	1′	
8 (sin-1)	1 (sin-1)		7 (sin-1)	2, 6, 9 (sin-1)	
2, 6 (sin-1)	4, 7 (sin-1)	3, 5 (sin-1)	7 (sin-2)	2, 6, 9 (sin-2)	
–OMe	3′, 5′ (sin-1)		2, 6 (sin-1)	4, 7 (sin-1)	3, 5 (sin-1)
2 (gal)	1 (glc-1)	1, 3 (gal)	2, 6 (sin-2)	4, 7 (sin-2)	3, 5 (sin-2)
3 (gal)		2 (gal)	8 (sin-1)	1 (sin-1)	
4 (gal)	2 (gal)	3 (gal)	8 (sin-2)	1 (sin-2)	
5 (gal)		6 (gal)	–OMe	3, 5 (sin-1, 2)	
1 (glc-1)	2 (gal)		2 (gal)	1 (glc-1)	1, 3 (gal)

Cont. overleaf

Table 3. (*Continued*)

3			4		
	C			C	
H	3-bond	2-bond	H	3-bond	2-bond
1 (glc-2)	4 (glc-1)		3 (gal)		2 (gal)
			4 (gal)	2 (gal)	
			1 (glc-2)	4 (glc-1)	
			2 (glc-2)	9 (sin-2)	1,3 (glc-2)
			3 (glc-2)		2,4 (glc-2)

Kaempferol 3-*O*-[6‴-*O*-*sinapoyl*-β-D-*glucopyranosyl*] (1 → 2)-β-D-*galactopyranoside* (**2**). A solid, $[\alpha]_D^{24}$ − 25.6° (MeOH; *c* 0.55), FABMS *m/z*: 839.2012 ($C_{38}H_{40}O_{20}Na$ requires 839.2010). UV λ_{max} nm (log ε): 240 (*sh*) (4.31), 266 (4.15), 329 (4.18). ^1H and ^{13}C NMR: see Tables 1 and 2.

Kaempferol 3-*O*-β-D-*glucopyranosyl*(1 → 4)[6‴-*O*-*sinapoyl*-β-D-*glucopyranosyl*](1 → 2)-β-D-*galactopyranoside* (**3**). A solid, $[\alpha]_D^{26}$ − 32.5° (MeOH; *c* 0.67), FABMS *m/z*: 1001.2546 ($C_{44}H_{50}O_{25}Na$ requires 1001.2538). UV λ_{max} nm (log ε): 240 (*sh*) (4.33), 266 (4.22), 330 (4.30). ^1H and ^{13}C NMR: see Tables 1–3.

Kaempferol 3-*O*-[2⁗-*O*-*sinapoyl*-β-D-*glucopyranosyl*] (1 → 4)[6‴-*O*-*sinapoyl*-β-D-*glucopyranosyl*](1 → 2)-β-D-*galactopyranoside* (**4**). A solid, $[\alpha]_D^{25}$ − 25.3° (MeOH; *c* 0.60), FAB MS *m/z*: 1207.3119 ($C_{55}H_{60}O_{29}Na$ requires 1207.3118). UV λ_{max} nm (log ε): 240 (*sh*) (4.49), 266 (4.34), 329 (4.44). ^1H and ^{13}C NMR: see Tables 1–3.

Quercetin 3-*O*-β-D-*glucopyranosyl*(1 → 2)-β-D-*galactopyranoside* (**5**). A solid, $[\alpha]_D^{30}$ − 15.5° (MeOH; *c* 0.38), FABMS *m/z*: 649.1379 ($C_{27}H_{30}O_{17}Na$ requires 649.1380). UV λ_{max} nm (log ε): 256 (4.28), 265 (*sh*) (4.23), 300 (*sh*) (3.86), 355 (4.14). ^1H and ^{13}C NMR: see Tables 1 and 2.

Quercetin 3-*O*-[6‴-*O*-*sinapoyl*-β-D-*glucopyranosyl*] (1 → 2)-β-D-*galactopyranoside* (**6**). A solid, $[\alpha]_D^{25}$ − 13.3° (MeOH; *c* 0.17), FABMS *m/z*: 855.1956 ($C_{38}H_{40}O_{21}Na$ requires 855.1960). UV λ_{max} nm (log ε): 242 (*sh*) (4.38), 256 (*sh*) (4.32), 265 (4.28), 332 (4.30). ^1H and ^{13}C NMR: see Tables 1 and 2.

Acknowledgement—We thank Mr H. Hanazono of Fukuoka University for MS measurements.

REFERENCES

1. Abe, F., Yamauchi, T. and Nohara, T. (1992) *Phytochemistry* **31**, 251.
2. Abe, F., Yamauchi, T. and Wan, A. S. C. (1992) *Phytochemistry* **31**, 3189.
3. Abe, F., Yamauchi, T., Yahara, S. and Nohara, T. (1994) *Phytochemistry* **37**, 1429.
4. Abe, F., Yamauchi, T., Yahara, S. and Nohara, T. (1994) *Phytochemistry* (in press).
5. Abe, F., Chen, R-F., Yamauchi, T. and Ohashi, H. (1994) *Chem. Pharm. Bull.* **43**, 499.
6. Nagy, E., Seres, I., Verzar-Petri, G. and Neszmelyi, A. (1984) *Z. Naturforsch.* **39B**, 1813.
7. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
8. Tantawy, B., Tollier, M. T. and Robin, J. P. (1986) *Bull. Liaison Group Polyphenols* **13**, 68.