PERGAMON

Phytochemistry 52 (1999) 147–151

PHYTOCHEMISTRY

Two acylated flavone glucosides from *Andrographis serpyllifolia*

A.G. Damu[a], B. Jayaprakasam[a], D. Gunasekar[a,*], A. Blond[b], B. Bodo[b]

[a]*Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati, 517 502, India*
[b]*Laboratoire de Chimie des Substances Naturelles, URA 401 CNRS, Museum National d'Histoire Naturelle, 63 rue Buffon, 75005, Paris, France*

Received 8 January 1999; accepted 10 February 1999

Abstract

Two new acylated flavone glucosides, skullcapflavone I 2′-*O*-*β*-D-(3″-*E*-cinnamoyl)glucopyranoside and skullcapflavone I 2′-*O*-*β*-D-(2″-*E*-cinnamoyl)glucopyranoside, together with skullcapflavone I 2′-*O*-*β*-D-glucopyranoside and andrographidine C have been isolated from the whole plant of *Andrographis serpyllifolia*. Structural elucidation of the glycosides was achieved by various NMR techniques including ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY experiments, FAB-mass spectrometry, acid hydrolysis and saponification.

Keywords: *Andrographis serpyllifolia*; Acanthaceae; Whole plant; Acylated flavone glucosides; Skullcapflavone I 2′-(3″-*E*-cinnamoyl)glucoside; Skullcapflavone I 2′-(2″-*E*-cinnamoyl)glucoside

1. Introduction

Andrographis serpyllifolia Wt.Ic (Acanthaceae) is a trailing and rooting procumbent herb widely distributed throughout Deccan and Carnatic regions of South India (Gamble, 1956). Previous chemical examination of the leaves, stems and roots of *A. serpyllifolia* afforded serpyllin, apigenin 7,4′-dimethyl ether and tectochrysin (Govindachari, Parthasarathy, Pai & Kalyanaraman, 1968). In the present communication we describe the isolation and structure elucidation of two new acylated flavone glucosides **1** and **2** in addition to skullcapflavone I 2′-glucoside (**3**) and andrographidine C (**4**).

2. Results and discussion

Compound **1**, obtained as a yellow amorphous powder, showed $[M+H]^{+}$ at m/z 607.1810 in its HRFABMS corresponding to the molecular formula $C_{32}H_{30}O_{12}$. This was corroborated by the ^{13}C NMR spectrum of **1**, which showed signals for all the 32 carbons of the molecule. The UV spectrum exhibited absorption maxima at 273, 321 (sh) and 352 (sh) nm, which was typical of flavones with 5,7,8-trioxygenation (Govindachari et al., 1968; Gupta, Taneja, Dhar & Atal, 1983; Giessman, 1962). Addition of sodium acetate did not cause any change in the band II absorption maximum indicating the absence of a free hydroxyl at C-7. The IR spectrum of **1** apart from hydroxyl (3393 cm^{-1}) and carbonyl (1616 cm^{-1}) absorption bands, showed an additional carbonyl absorption band at 1678 cm^{-1} indicating the presence of an ester group conjugated with a double bond (Karl, Muller & Pedersen, 1976; Aritomi, 1963).

The ^{1}H NMR spectrum of **1** showed a downfield signal at δ 12.68, exchangeable with D_2O, which could be attributed to a chelated C-5 hydroxyl group. A sharp singlet at δ 6.59 was assigned to H-6 as it showed correlation with C-5 (156.6 ppm) in its HMBC spectrum (Fig. 1). It also exhibited two methoxyl signals at δ 3.91 and 3.80, and the former methoxyl group was assigned to C-7 as it showed long range correlation with H-6 in its ROESY spectrum (Fig. 1). The other methoxyl group at δ 3.80 was placed at C-8 as it resonated at 61.1 ppm, a chemical shift character-

* Corresponding author. Tel.: +91-8574-50666; fax: +91-8574-27499.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00113-2

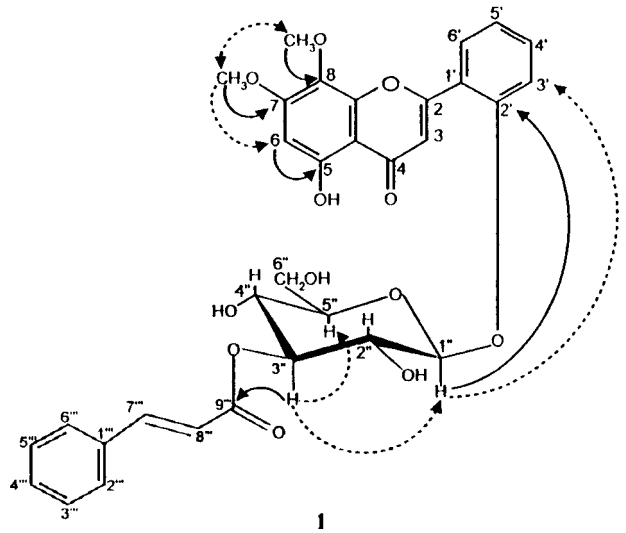


Fig. 1. Significant HMBC ($^{2\text{-}3}J_{C11}$) (→) and ROESY (⇢) correlations for compound **1**.

istic of a di-*ortho* substituted methoxyl group (Iinuma, Matsuura & Kusuda, 1980). The presence of a sharp singlet at δ 7.01 was assigned to H-3 based on ^{13}C-^{1}H HMQC evidence. It also displayed the characteristic pattern of a 2′-oxygenated ring B (Kuroyanagi, Sato, Ueno & Nishi, 1987) with signals at δ 7.87 (*dd, J* = 7.9, 1.7 Hz, 1H), 7.56 (*ddd, J* = 8.5, 8.0, 1.7 Hz, 1H), 7.42 (*m*, 1H) and 7.26 (*ddd, J* = 8.0, 7.9, 0.9 Hz, 1H), which were assigned to the 6′, 4′, 3′ and 5′ protons, respectively.

An anomeric proton signal at δ 5.35 (*d, J* = 7.9 Hz) suggested the presence of a sugar residue with β-configuration. The remaining signals at δ 6.67 (*d, J* = 16 Hz, 1H), 7.68 (*d, J* = 16 Hz, 1H), 7.42 (*m*, 3H) and 7.73 (*m*, 2H) indicated a *trans*-cinnamoyl moiety in **1** and its presence was further evidenced by the formation of *trans*-cinnamic acid, D-glucose and an aglycone, skullcapflavone I (**1a**) (Jalal, Overton & Rycroft, 1979) when **1** was subjected to acid hydrolysis. The glucose residue in **1** was found to be linked to C-2′ since a NOE was observed between H-1″ and H-3′ in its ROESY spectrum and a cross peak between H-1″ and C-2′ was seen in its HMBC spectrum (Fig. 1).

Alkaline hydrolysis of **1** gave *trans*-cinnamic acid and skullcapflavone I 2′-*O*-β-D-glucoside (**3**) (Gupta, Taneja & Dhar, 1996) indicating that the cinnamoyl moiety was attached to the glucosyl residue. The cinnamoyl residue in **1** was found to be linked to the C-3″ hydroxyl of the glucose as this carbon signal was shifted downfield (Dorman & Roberts, 1970; Kamerling, De Bie & Vliegenthart, 1972; Yamasaki et al., 1977; Agrawal & Bansal, 1989) by 1.40 ppm, while the C-2″ and C-4″ signals were shifted upfield by 1.70 and 2.0 ppm, respectively compared with **3** (Table 1). The site of esterification in **1** was confirmed by a downfield shift (Markham & Geiger, 1994) of 1.78 ppm observed for H-3″ (δ 5.08, *dd, J* = 9.2, 9.2 Hz) compared with that of **3** (δ 3.30, *dd, J* = 9.0, 9.0 Hz). The attachment of cinnamoyl moiety at C-3″ in **1** was further supported by the presence of a cross peak between H-3″ (δ 5.08) of the glucose and carbonyl carbon (165.7 ppm) of the cinnamoyl residue in its HMBC spectrum (Fig. 1) and two strong NOEs observed for H-3″ (δ 5.08) with H-1″ (δ 5.35) and H-5″ (δ 3.55) in its ROESY spectrum (Fig. 1). On the basis of the foregoing studies, the structure of **1** was established as skullcapflavone I 2′-*O*-β-D-(3″-*E*-cinnamoyl)-glucopyranoside (**1**).

Compound **2**, obtained as yellow amorphous powder, showed a $[M+H]^+$ peak at *m/z* 607.1807 in its HRFABMS consistent with the molecular formula $C_{32}H_{30}O_{12}$. The UV absorption maxima of **2** (268, 300 (sh), 348 (sh) nm) resembled that of **1** indicating the presence of an identical flavone skeleton to that of **1**. The IR spectrum was characterised by a strong carbonyl ester band at 1710 cm^{-1}, in addition to the usual carbonyl band of flavone at 1609 cm^{-1} and hydroxyl absorption band at 3457 cm^{-1}. The acid and alkaline

Table 1
^{13}C NMR spectral data (ppm) of compounds **1**–**3** (75 MHz, DMSO-d_6)

C	1	2	3
2	161.0	160.8	160.8
3	110.2	110.4	110.1
4	182.3	181.7	182.2
4a	103.9	103.8	103.8
5	156.6	156.5	156.5
6	95.9	95.7	95.8
7	158.5	158.1	158.3
8	128.5	128.2	128.3
8a	149.1	149.0	149.0
1′	120.4	121.5	120.1
2′	155.2	155.1	155.4
3′	115.6	116.3	115.4
4′	133.1	132.9	132.9
5′	122.3	122.8	122.0
6′	129.0	129.1	128.7
7-OMe	56.5	56.1	56.3
8-OMe	61.1	60.6	61.0
1″	99.6	99.5	99.9
2″	71.4[a]	73.2	73.1
3″	77.9	73.6	76.5
4″	67.4[a]	69.8	69.4
5″	76.8	77.5	77.0
6″	60.2	60.5	60.4
1‴	134.2	133.4	–
2‴, 6‴	128.3	128.0	–
3‴, 5‴	129.0	128.5	–
4‴	130.3	130.2	–
7‴	144.2	144.5	–
8‴	118.7	117.0	–
9‴	165.7	164.8	–

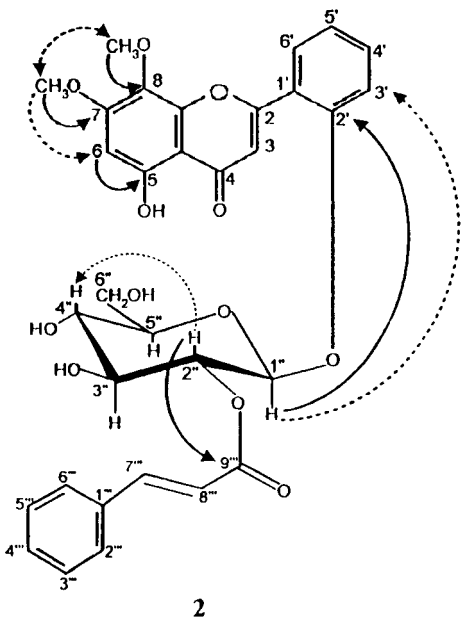
[a] Assignments exchangeable.

hydrolysis products of **2** were found to be identical with those obtained from **1**. From the molecular formula and from the nature of the products obtained in acid and alkaline hydrolysis, **2** should be an isomeric glycoside of **1** differing in the point of attachment of the cinnamoyl moiety to the glucose unit.

The ^{13}C NMR spectrum of **2** was similar to **1** (Table 1) except in the carbon resonances of the glucose unit. A close comparison of the sugar carbon signals in **2** with that of **3** (Table 1) showed that the cinnamoyl moiety in **2** was located at C-2″ as this carbon signal was deshielded by 0.1 ppm whereas the C-1″ and C-3″ signals had moved upfield by 0.4 and 2.9 ppm, respectively. Also, in the ^1H NMR spectrum, the chemical shift for H-2″ (δ 4.94, *dd, J* = 9.5, 8.1 Hz) was indicative of acylation at C-2″ of the glucose moiety (Birkofer, Kaiser, Hillges & Becker, 1969). The site of acylation was further supported by the HMBC correlation between the carbonyl carbon (164.8 ppm) of the cinnamoyl moiety and H-2″, and a strong NOE observed for H-2″ (δ 4.94) with H-4″ (δ 3.31) in its ROESY spectrum (Fig. 2). Thus, **2** was characterised as skullcapflavone I 2′-*O*-*β*-D-(2″-*E*-cinnamoyl)glucopyranoside.

Compounds **3** and **4** were identified as skullcapflavone I 2′-*O*-*β*-D-glucopyranoside and andrographidine C, respectively by acid hydrolytic studies and by direct comparison of their spectral data with those earlier reported from *A. paniculata* (Kuroyanagi et al., 1987; Gupta et al., 1996).

The occurrence of **1** and **2** constitutes the first report of acylated flavone glycosides in the genus *Andrographis*.



2

Fig. 2. Significant HMBC ($^{2\text{-}3}J_{C11}$) (→) and ROESY (⇢) correlations for compound **2**.

3. Experimental

3.1. General

Mps were uncorr. IR spectra were recorded in KBr. ^1H and ^{13}C NMR spectra were determined on a Bruker AC 300 spectrometer operating at 300.13 and 75.43 MHz, respectively using DMSO-d_6 with TMS as int. standard. ^1H–^1H COSY, HMQC, HMBC and the phase-sensitive ROESY (with 150 ms mixing time) spectra were recorded using the standard pulse sequences. FAB and HRFAB mass spectra were obtained on a 700 JEOL mass spectrometer in thioglycerol matrix. CC was performed on Acme silica gel finer than 200 mesh (0.08 mm). PC was carried out on Whatman No 1 using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5).

3.2. Plant material

The whole plant of *A. serpyllifolia* Wt.Ic was collected in March 1996 at Talakona Hills, Andhra Pradesh, India. A voucher specimen (DG-198) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

3.3. Extraction and isolation

Dried and powdered whole plant (2.5 kg) was successively extracted with hexane, Me_2CO and MeOH. The Me_2CO extract was concentrated in vacuo to give a brown viscous residue. It was defatted with *n*-hexane and C_6H_6. The residual brown solid (66 g) was subjected to CC on silica gel and eluted with $CHCl_3$, $CHCl_3$–EtOAc and EtOAc–MeOH mixtures. $CHCl_3$–EtOAc 1:1 and 2:8 frs yielded **1** (30 mg) and **2** (40 mg), respectively. EtOAc–MeOH 9:1 and 8:2 frs gave **3** (45 mg) and **4** (50 mg), respectively.

3.3.1. Skullcapflavone I 2′-*O*-*β*-D-(2″-*E*-cinnamoyl)glucopyranoside (*1*)

Yellow amorphous powder (MeOH); mp 236–237°; $[\alpha]_D^{25}$ −0.09° (MeOH, *c* 5.0); HRFABMS (positive mode) *m/z*: 607.1810 ($[M+H]^+$; $C_{32}H_{30}O_{12}+H$ requires 607.1815); FABMS (positive mode) *m/z* (rel. int): 607 $[M+H]^+$ (32), 477 $[M+H-$cinnamoyl$]^+$ (2), 315 $[M+H-$cinnamoyl glucosyl$]^+$ (53); UV λ_{max}^{MeOH} nm (log ε): 273 (4.84), 321 sh (4.52), 352 sh (4.36); (NaOMe) 275, 385; (NaOAc) 272, 320 sh, 352 sh; (NaOAc + H_3BO_3) 272, 320 sh, 352 sh; ($AlCl_3$) 280, 335, 392 sh; ($AlCl_3$ + HCl) 273, 330 sh, 360; IR ν_{max}^{KBr} cm^{-1}: 3393 (OH), 1678 (C═O), 1616 (C═O), 1570,

1520; ^{1}H NMR (DMSO-d_6): δ 12.68 (1H, *s*, OH-5), 7.87 (1H, *dd, J* = 7.9, 1.7 Hz, H-6′) 7.73 (2H, *m*, H-2‴, 6‴), 7.68 (1H, *d, J* = 16.0 Hz, H-7‴), 7.56 (1H, *ddd, J* = 8.5, 8.0, 1.7 Hz, H-4′), 7.42 (4H, *m*, H-3′, 3‴, 4‴, 5‴), 7.26 (1H, *ddd, J* = 8.0, 7.9, 0.9 Hz, H-5′) 7.01 (1H, *s*, H-3), 6.67 (1H, *d, J* = 16.0 Hz, H-8‴) 6.59 (1H, *s*, H-6), 5.35 (1H, *d, J* = 7.9 Hz, H-1″), 5.08 (1H, *dd, J* = 9.2, 9.2 Hz, H-3″), 3.91 (3H, *s*, OMe-7), 3.80 (3H, *s*, OMe-8), 3.71 (1H, *m*, H-6″a), 3.55 (4H, *m*, H-2″, 4″, 5″, 6″b); ^{13}C NMR in Table 1.

3.3.2. Skullcapflavone I 2′-*O*-β-D-(2″-*E*-cinnamoyl)glucopyranoside (2)

Yellow amorphous powder (MeOH); mp 196–197°, $[\alpha]_D^{25}$ −0.15° (MeOH, *c* 4.0); HRFABMS (positive mode) *m/z*: 607.1807 ($[M+H]^+$; $C_{32}H_{30}O_{12}+H$ requires, 607.1815); FABMS (positive mode) *m/z* (rel. int): 607 $[M+H]^+$ (100), 477 (1), 315 (44); UV λ_{max}^{MeOH} nm (log ε): 268 (4.45), 300 sh (3.94), 348 sh (3.51); (NaOMe) 278, 380; (NaOAc) 268, 300 sh, 348; (NaOAc + H_3BO_3) 268, 300 sh, 348; ($AlCl_3$) 275, 325 sh, 362; ($AlCl_3$ + HCl) 265, 270, 305, 360; IR ν_{max}^{KBr} cm^{-1}: 3457 (OH), 1710 (C═O ester), 1609 (C═O), 1586, 1511, 1448; ^{1}H NMR (DMSO-d_6): δ 12.68 (1H, *s*, OH-5), 7.66 (1H, *dd, J* = 7.5, 1.8 Hz, H-6′), 7.57 (1H, *ddd, J* = 8.5, 7.5, 1.8 Hz, H-4′), 7.41 (1H, *dd, J* = 8.5, 0.9 Hz, H-3′), 7.38 (2H, *m*, H-2‴, 6‴), 7.35 (1H, *m*, H-4‴), 7.31 (2H, *m*, H-3‴, 5‴), 7.23 (1H, *ddd, J* = 7.5, 7.5, 0.9 Hz, H-5′), 7.21 (1H, *d, J* = 16.0 Hz, H-7‴), 6.41 (2H, *s*, H-3, 6), 6.32 (1H, *d, J* = 16.0 Hz, H-8‴), 5.21 (1H, *d, J* = 8.1 Hz, H-1″), 4.94 (1H, *dd, J* = 9.5, 8.1 Hz, H-2″), 3.80 (3H, s, OMe-7), 3.77 (1H, *m*, H-6″a), 3.57 (3H, s, OMe-8), 3.53 (3H, *m*, H-3″, 5″, 6″b), 3.31 (1H, *m*, H-4″); ^{13}C NMR in Table 1.

3.3.3. Skullcapflavone I 2′-*O*-β-D-glucopyranoside (3)

Yellow needles (MeOH); mp 260–262°; FABMS (positive mode) *m/z* (rel. int.): 477 $[M+H]^+$ (46), 315 $[M+H-glucosyl]^+$ (100); ^{13}C NMR in Table 1; MS, UV, IR and ^{1}H NMR data were identical with published data (Gupta et al., 1996).

3.3.4. Andrographidine C (4)

Pale yellow needles (MeOH); mp 115–116°, FABMS (positive mode) *m/z* (rel. int): 461 $[M+H]^+$ (46); 299 $[M+H-glucosyl]^+$ (100); MS, UV, IR and ^{1}H and ^{13}C NMR data were identical with published data (Kuroyanagi et al., 1987).

3.4. Acid hydrolysis of compounds 1 and 2

10 mg of the glycoside was refluxed with 2N HCl (2.5 ml) in MeOH for 2 h. The yellow ppt was filtered and recryst. from MeOH to give pale yellow needles of skullcapflavone I (3 mg), mp 254–255°. The filtrate was extracted with EtOAc in which *trans*-cinnamic acid was identified by co-PC (BAW). The sugar in the aq. layer was identified as D-glucose by co-PC (BAW).

3.5. Alkaline hydrolysis of compounds 1 and 2

A soln of the glycoside (5 mg) in 1% KOH (5 ml) was refluxed for 2 h. The reaction mixt was acidified with 1N HCl and extracted with Et_2O followed by *n*-BuOH. The Et_2O extract was washed with H_2O, evapd to dryness, redissolved in MeOH (0.5 ml) and submitted to PC when *trans*-cinnamic acid was identified, while the residue obtained from the *n*-BuOH extract was cryst. from MeOH to yield skullcapflavone I 2′-*O*-β-D-glucoside (4 mg), mp. 260–262°.

Acknowledgements

BJ acknowledges Council of Scientific and Industrial Research, New Delhi for financial assistance. We are grateful to Mr J. P. Brouard, Laboratoire de Chimie des Substances Naturelles, MNHN, Paris and Michel Becchi, Centre de Spectrométrie de masse du, CNRS, Lyon, France for providing mass spectral data.

References

Agrawal, P. K., & Bansal, M. C. (1989). Flavonoid glycosides. In P. K. Agrawal, *Carbon-13 NMR of flavonoids* (p. 313). Amsterdam: Elsevier.

Aritomi, M. (1963). Terniflorin, a new flavonoid compound in flowers of *Clematis terniflora* var. robusta. *Chemical and Pharmaceutical Bulletin*, *11*, 1225.

Birkofer, L., Kaiser, C., Hillges, B., & Becker, F. (1969). Sugar esters V. NMR spectroscopic studies of o-acyl glycoses. *Annals of Chemistry*, *725*, 196.

Dorman, D. E., & Roberts, J. D. (1970). NMR spectroscopy. C-13 spectra of some pentose and hexose aldopyranoses. *Journal of American Chemical Society*, *92*, 1355.

Gamble, J. S. (1956). Flora of the presidency of Madras, vol. 2 (p. 1049). Calcutta: Botanical Survey of India.

Giessman, T. A. (1962). Spectral properties of flavonoid compounds. In *The chemistry of flavonoid compounds* (p. 111). London: Pergamon Press.

Govindachari, T. R., Parthasarathy, P. C., Pai, B. R., & Kalyanaraman, P. S. (1968). Chemical investigation of *A. sepyllipolia*: isolation and structure of serpyllin, a new flavone. *Tetrahedron*, *24*, 7027.

Gupta, K. K., Taneja, S. C., & Dhar, K. L. (1996). Flavonoid glycoside of *A. paniculata*. *Indian Journal of Chemistry*, *35B*, 512.

Gupta, K. K., Taneja, S. C., Dhar, K. L., & Atal, C. K. (1983). Flavonoids of *A. paniculata*. *Phytochemistry*, *22*, 314.

Iinuma, M., Matsuura, S., & Kusuda, K. (1980). ^{13}C NMR spectral studies on polysubstituted flavonoid. I. C-13 MNR spectra of flavones. *Chemical and Pharmaceutical Bulletin*, *28*, 708.

Jalal, M. A. F., Overton, K. H., & Rycroft, D. S. (1979). Formation of three new flavones by differentiating callus cultures of *A. Paniculata*. *Phytochemistry*, *18*, 149.

Kamerling, J., De Bie, M., & Vliegenthart, J. (1972). A PMR study of the anomeric protons in pertrimethylsilyl oligosaccharides, a

determination of the configuration of the glycosidic bond. *Tetrahedron*, *28*, 3037.

Karl, C., Muller, G., & Pedersen, P. A. (1976). Flavonoide aus salix alba. Die struktur des terniflorins und weiteren acyl flavonoides. *Phytochemistry*, *15*, 1084.

Kuroyanagi, M., Sato, M., Ueno, A., & Nishi, K. (1987). Flavonoids from *A. panicalata*. *Chemical and Pharmaceutical Bulletin*, *35*, 4429.

Markham, K. R., & Geiger, H. (1994). ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulphoxide. In J. B. Harborne, *The flavonoids: Advances in research since 1986* (p. 467). London: Chapman & Hall.

Yamasaki K., Kasai R., Masaki Y., Okihara M., Tanaka H., Takagi S., Yamaki M., Masuda K., Nonaka G., Tsuboi M., Nishioka I. (1977). Application of C-13 NMR to the structural elucidation of acylated plant glycosides. *Tetrahedron Letters* 1231.