Phytochemistry, Vol. 31, No. 4, pp. 1277–1280, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

SECOIRIDOID, COUMARIN AND SECOIRIDOID-COUMARIN GLUCOSIDES FROM *FRAXINUS CHINENSIS*

HIROSHI KUWAJIMA, MASAMI MORITA, KIYOKAZU TAKAISHI, KENICHIRO INOUE,* TETSURO FUJITA,* ZHENG-DAN HE and CHONG-REN YANG†

Faculty of Pharmaceutical Sciences of Kinki University, Higashi-osaka 577, Japan; *Faculty of Pharmaceutical Sciences of Kyoto University, Sakyou-ku, Kyoto 606, Japan; †Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China

(*Received* 9 *July* 1991)

Key Word Index—*Fraxinus chinensis*; Oleaceae; secoiridoid glucoside; frachinoside.

Abstract—Besides the known glucosides, oleuropein, neooleuropein and cichoriin, a new secoiridoid glucoside, frachinoside, was isolated from the leaves of *Fraxinus chinensis* and its structure elucidated.

INTRODUCTION

Oleaceae is a rich source of secoiridoid and phenylpropanoid glucosides. A number of secoiridoid glucosides including oleuropein (**1**), ligstoside (**2**) and nuezhenide (**3**) have been reported from the genus *Fraxinus* [1, 2]. In the course of phytochemical and biosynthetic studies on secoiridoid glucosides in oleaceous plants [3], we have now investigated the constituents of the leaves of *F. chinensis* Roxb. (Chinese name, Bai la shu) and isolated a new secoiridoid glucoside together with three known glucosides.

RESULTS AND DISCUSSION

The water soluble part of the methanolic extract of the fresh leaves of *F. chinensis* (Experimental) gave on column chromatography and further purification through preparative TLC, a new secoiridoid glucoside, frachinoside (**4**), in addition to oleuropein (**1**), neooleuropein (**5**) [4] and a coumarin glucoside, cichoriin (**6**) [5].

The glucoside (**5**) was obtained as a powder, $C_{32}H_{38}O_{15}$, $[\alpha]_D^{15}$ −130.43° (MeOH). It showed UV maxima at 230 and 283 nm (log ε 4.31, 3.82) and IR bands at 3350, 1700, 1690, 1620 and 1520 cm^{-1}, which suggested that **5** is a secoiridoid glucoside related to **1** and **2** with aromatic properties. Its ^1H (Table 1) and ^{13}C (Table 2) NMR spectra very closely resembled those of oleuropein (**1**). The only difference being that **5** had signals due to one more 3,4-dihydroxyphenethyl group instead of a signal of the carbomethoxy group present in **1**. This finding was supported by the positive FAB mass spectrum which showed a quasimolecular ion peak $[M+H]^+$ at *m/z* 663, indicating an increase of 122 mass units in comparison with that of **1**. These features suggested that **5** is a glucoside in which the carbomethoxy group of **1** was replaced with a 3,4-dihydroxyphenethyl group, i.e. neooleuropein. The physical and spectral data of the octaacetate of **5** were in full agreement with those reported for neooleuropein octaacetate (**5a**) [4]. The absolute structure of **5** was, therefore, established. Although this glucoside had already been obtained as its acetate from the

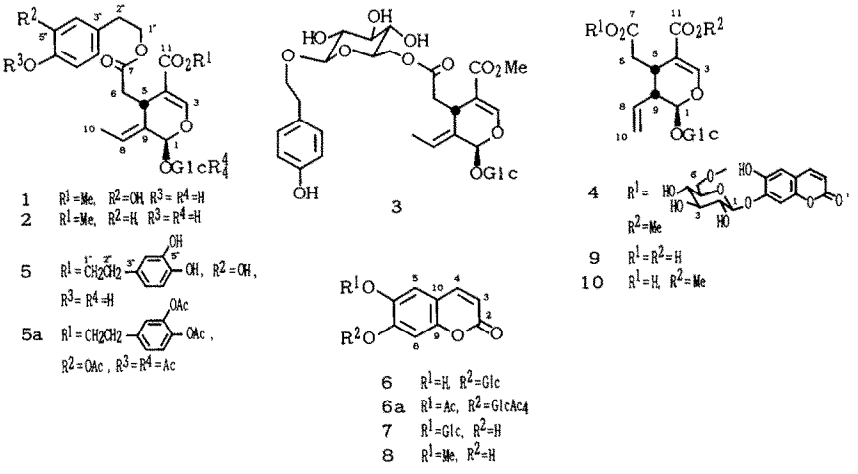

Table 1. ^{1}H NMR spectral data of glucosides **1**, **4**–**6** and **10** (in CD_3OD, at 500 MHz)

		4 (600 MHz)*				
H	**1**	**A**	**B**	**5**	**6** (300 MHz)	**10** (in D_2O)
1	5.90 *quint like* (1.5)	5.44 *d* (3.9)		5.94 *quint like* (1.5)		5.53 *d* (5.0)
3	7.51 *s*	7.44 *d* (1.8)	6.27 *d* (9.5)	7.52 *s*	6.28 *d* (9.5)	7.51 *br s*
4			7.81 *d* (9.5)		7.82 *d* (9.5)	
5	3.96 *dd* (9.0, 4.5)	3.34–3.39 *m*	7.18 *s*	3.98 *dd* (9.5, 4.5)	7.20 *s*	3.22 *m*
6a	2.44 *dd* (14.0, 9.0)	2.34 *dd* (16.2, 8.5)	—	2.43 *dd* (14.0, 9.5)	—	2.58 *br dd* (15.5, 5.5)
6b	2.70 *dd* (14.0, 4.5)	3.06 *dd* (16.2, 5.9)	—	2.70 *dd* (14.0, 4.5)	—	2.77 *br dd* (15.5, 8.5)
8	6.08 *qd* (7.0, 1.5)	5.56 *dt* (17.0, 10.0)	7.02 *s*	6.12 *qd* (7.0, 1.5)	7.04 *s*	5.71 *br dt* (17.0, 10.0)
9		2.84 *ddd* (10.0, 5.6, 3.9)				2.77 *m*
10a	1.66 *dd* (7.0, 1.5)	4.97 *dd* (10.0, 1.4)		1.70 *dd* (7.0, 1.5)		5.29 *br d* (10.0)
10b	—	5.04 *dd* (17.0, 1.4)		—		5.34 *br d* (17.0)
OMe	3.71 *s*	3.58 *s*				3.74 *s*
1′	4.80 *d* (8.0)	4.67 *d* (7.9)	5.01 *d* (7.8)	4.85 *d* (8.0)	4.98 *d* (7.5)	4.84 *d* (8.5)
2′		3.28 *dd* (9.4, 7.9)	3.67 *dd* (9.2, 7.8)		3.55 *dd* (9.3, 8.7)	3.33 *dd* (9.0, 8.5)
3′		3.34–3.39 *m*	3.54 *t* (9.2)		3.52 *dd* (9.3, 8.7)	3.52 *t like* (9.0)
4′		3.34–3.39 *m*	3.45 *dd* (9.8, 9.2)		3.42 *dd* (9.7, 8.7)	3.42 *t like* (9.5)
5′		3.34–3.39 *m*	3.79 *ddd* (9.8, 6.6, 2.2)		3.53 *ddd* (9.8, 5.7, 2.3)	3.48–3.55 *m*
6′a	3.67 *dd* (12.0, 5.5)	3.72 *dd* (12.0, 5.3)	4.11 *dd* (12.0, 6.6)	3.72 *dd* (12.0, 5.5)	3.73 *dd* (12.1, 5.7)	3.75 *dd* (12.0, 6.0)
6′b	3.88 *dd* (12.0, 2.0)	3.88 *dd* (12.0, 2.3)	4.69 *dd* (12.0, 2.2)	3.94 *dd* (12.0, 1.5)	3.93 *dd* (12.1, 2.3)	3.93 *dd* (12.0, 2.0)
1″	4.10, 4.20 each *dt* (11.0, 7.0)			4.15, 4.26, 4.31, 4.35 each *dt* (11.0, 7.0)		
2″	2.76 *t* (7.0)			2.82, 2.87 each *t* (7.0)		
4″	6.66 *d* (2.0)			6.72, 6.74 each *d* (2.5)		
7″	6.69 *d* (8.0)			6.74, 6.75 each *d* (8.0)		
8″	6.54 *dd* (8.0, 2.0)			6.60, 6.61 each *dd* (8.0, 2.5)		

Assignments of the signals of **4** were made by ^{1}H–^{1}H COSY spectrum.
*A: secoxyloganin part; **B**: cichoriin part.

Table 2. ^{13}C NMR spectral data of glucosides **1**, **4**–**7** and **10** (in CD_3OD, at 125.65 Hz)

		4 (75.46 Hz)*					
C	1	A	B	5	6	7	10
1	95.21 *d*	97.78	—	95.25	—	—	97.55
2	—	—	163.43 *s*	—	163.74 *s*	163.63 *s*	—
3	155.14 *d*	153.79	114.84	155.13	114.58	113.16	153.59
4	109.39 *s*	109.89	145.46 *d*	109.55	145.64 *d*	145.87 *d*	110.07
5	31.80 *d*	28.66	113.82	31.74	114.01	117.16	28.56
6	41.25 *t*	35.69	145.32 *s*	41.17	145.64 *s*	144.37 *s*	35.06
7	173.21 *s*	174.05	150.00	173.25	150.78	153.39	176.20
8	124.87 *d*	134.33	105.24	124.88	105.35	104.62	134.41
9	130.74 *s*	45.07 *d*	149.63	130.42	149.32	152.70	45.29 *d*
10	13.55 *q*	120.65 *t*	115.17 *s*	13.55	115.35 *s*	112.88 *s*	120.57 *t*
11	168.69 *s*	168.86		168.24			168.88
OMe-11	51.92 *q*	51.71		—			51.65
1′	100.88 *d*	100.11	102.39	100.89	103.12	104.52	99.94
2′	74.74 *d*	74.52	74.48	74.74	74.70	74.86	74.58
3′	78.40 *d*	78.15[a]	77.30	78.35	78.48	78.51	78.34
4′	71.45 *d*	71.16	71.56	71.45	71.29	71.47	71.50
5′	77.91 *d*	77.98[a]	75.63	77.93	77.46	77.71	77.94
6′	62.71 *t*	62.31	64.27	62.71	62.38	62.62	62.71
1″	66.88 *t*			66.88, 66.34			
2″	35.38 *t*			35.36, 35.48			
3″	130.48 *s*			130.77, 130.99			
4″	117.06 *d*			117.09, 117.02			
5″	146.22 *s*			146.24, 146.19			
6″	144.91 *s*			144.88, 144.88			
7″	116.43 *d*			116.48, 116.39			
8″	121.31 *d*			121.31, 121.36			

[a]Values are interchangeable.

*A: secoxyloganin part; **B**: cichoriin part.

The signals of glucoside **4** were assigned on the basis of ^{13}C–^{1}H COSY and DEPT spectra; the others were made by gated decoupling mode. Multiplicities are not repeated if identical with those of **1**.

leaves of *Syringa vulgaris* [4], this is the first time it has been isolated as the native form.

The glucoside (**6**) was obtained as needles, mp 210°, $C_{15}H_{16}O_9$, $[\alpha]_D^{15}$ −97.60° (50% MeOH). It showed UV absorption at 227, 255, 290 and 345 (log ε 4.07, 3.59, 3.69, 3.77), IR bands at 3300, 1710sh, 1690, 1680sh and 1570 cm^{-1}, and ^{1}H NMR signals (Table 1) typical of 6,7-dihydroxycoumarin glucosides [6]. These features coupled with its ^{13}C NMR spectrum (Table 2) and molecular formula suggested that it should be either cichoriin (**6**) or esculin (**7**). Methylation of **6** followed by acidic hydrolysis gave scopoletin (**8**), identical with an authentic sample, and glucose. The β-nature of the sugar linkage was evident from the coupling constant (J = 8.0 Hz) of the anomeric proton. Thus, glucoside (**6**) proved to be cichoriin which had been also isolated from *Fraxinus ornus* [5].

Frachinoside (**4**), $C_{32}H_{38}O_{19}$, was obtained as a powder of $[\alpha]_D^{16}$ −114.10° (MeOH). It showed UV absorption at 227, 287 and 343 nm (log ε 4.36, 3.79, 3.86), IR bands at 3400, 1720sh, 1710, 1690sh, 1630, 1620sh and 1560 cm^{-1}, and ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) with all signals characteristic of a secologanoside (**9**) type secoiridoid glucoside and cichoriin (**6**). Confirmative evidence of the position of attachment of these glucosides was provided by NMR data as follows: the lower field shifts (δ 4.11, 4.69) of the C-6′ methylene protons of the cichoriin (**6**) moiety in **4** when compared to the corresponding signals at δ 3.63 and 3.82 in **6** suggested that one of the two carboxyl groups of **9** is linked by an ester bond to this carbon. This was supported by its ^{13}C NMR data, which showed C-6′ and C-5′ signals of the cichoriin (**6**) moiety resonating at δ 64.27 and 75.63 in **4**, and at 62.38 and 77.46 in **6**, respectively. In addition, the ^{1}H NMR spectrum of **4** showed a signal due to a carbomethoxy group at δ 3.58 (3H, *s*), indicating the existence of a methyl ester in this molecule.

To confirm the attached positions of the methyl group and cichoriin (**6**) moiety to the secoiridoid moiety, **4** was subjected to partial hydrolysis with sodium hydroxide (0.2 M), and yielded **6** and secoxyloganin (**10**). The identity of the latter was confirmed from the NMR and $[\alpha]_D$ data already reported [7], and thus, the structure of frachinoside was elucidated as cichoriinylsecoxyloganin (**4**). Generally, a carbomethoxy group conjugated with an iridoid enol system as shown in **10** would appear give rise to a ^{1}H NMR signal at about δ 3.70 [7]. The higher field shift of the carbomethoxy group in **4** compared to that of **10** would be caused by the anisotropic effect of the coumarin skeleton.

Frachinoside (**4**) is the first example of a novel secoiridoid linked to a coumarin glucoside, although oleuropein

(**1**) and esculin (**7**) do co-exist in *F. japonica* [1]. This is the second example of a secologanoside (**9**) type secoiridoid glucoside to be isolated from this family [8].

EXPERIMENTAL

General. Mps: uncorr; NMR: ^1H, 300, 500, 600 MHz, ^{13}C, 125.65, 75.46 MHz, TMS as int. standard; TLC: silica gel GF_{254}, spots visualized by irradiation under UV light (254 nm), by exposure to I_2 vapour or by spraying with anisaldehyde–H_2SO_4 reagent and heating; prep. TLC: silica gel PF_{254}, bands detected under UV light or by exposure to I_2 vapour; CC: highly porous polymer Diaion HP-21 (Mitsubishi Kasei) and silica gel (Merck); GLC: detect, FID, flow rate, N_2 40 ml min^{-1}, column dimension, 200 × 3 mm, packing, 3% SE-52.

Plant material. Leaves of *Fraxinus chinensis* grown in the Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences were collected in October 1990.

Isolation of glucosides. The fresh leaves (2.34 kg) of *F. chinensis* were extracted with hot MeOH (20 l × 4) and the residue obtained by removal of the solvent *in vacuo* was triturated with H_2O (2 l). The insoluble material was filtered off through a Celite layer, which was washed with H_2O (900 ml), and the filtrate and washings were combined and concd *in vacuo* to *ca* 500 ml; the aq. soln was extracted with *n*-BuOH (300 ml × 4). The *n*-BuOH layer was concd *in vacuo* to give a residue (89.0 g), which was subjected to CC on the highly porous polymer HP-21 (1783 ml), eluting with H_2O–MeOH of increasing MeOH content. The eluates with 50% H_2O–MeOH (4 l) and MeOH (4 l) were concd *in vacuo* to give residues R-1 (35.08 g) and R-2 (48.10 g), respectively. An aliquot of R-1 (200 mg) was purified by prep. TLC ($CHCl_3$–MeOH–HOAc, 40:10:1, 2 developments) followed by recrystallization (H_2O–MeOH) to give cichoriin (**6**) (40 mg). An aliquot of R-2 (10 g) was chromatographed on a silica gel (300 g) column with $CHCl_3$–MeOH with increasing MeOH content. The frs eluted with $CHCl_3$–MeOH (185:15), (9:1) and (4:1) were concd *in vacuo* to afford residues R-2/1 (1.247 g), R-2/2 (370 mg) and R-2/3 (1.294 g), respectively. An aliquot of R-2/1 (570 mg) was subjected to prep. TLC (1st: $CHCl_3$–MeOH–HOAc, 40:10:1, 3 developments, 2nd: C_6H_6–EtOAc–EtOH, 1:4:1, 2 developments) to give oleuropein (**1**) (38.1 mg) as a powder. R-2/2 (370 mg) was subjected to prep. TLC (1st: $CHCl_3$–MeOH–HOAc, 40:10:1, 3 developments; 2nd: $CHCl_3$–MeOH, R_f 0.35) to give neooleuropein (**5**) (24.7 mg). On further purification by prep. TLC (1st: $CHCl_3$–MeOH, 4:1, 2 developments, 2nd: EtOAc–MeOH–H_2O, 200:33:27, 3 developments; 3rd: Me_2CO–$CHCl_3$–H_2O, 32:8:1, 2 developments) of R-2/3 (556.7 mg) gave frachinoside (**4**) (40.8 mg).

Neooleuropein (**5**). $[\alpha]_D^{15}$ −130.43° (MeOH; *c* 0.506); UV λ_{max}^{MeOH} nm (log ε): 230 (4.31), 282 (3.82); IR ν_{max}^{KBr} cm^{-1}: 3350, 1700, 1690, 1620, 1520, FAB-MS *m/z* 663 $[M+H]^+$. (Found: C, 57.7; H, 5.6. Calcd. for $C_{32}H_{38}O_{15}$: C, 58.0; H, 5.8%.) Compound **5** (10.5 mg) was acetylated with pyridine–Ac_2O (each 0.2 ml) in the usual way and the product (16.4 mg) purified by prep. TLC ($CHCl_3$–MeOH, 49:1) to give neooleuropein octaacetate (**5a**) (14.5 mg) as a powder. $[\alpha]_D^{16}$ −89.23° ($CHCl_3$; *c* 1.30, ref. [4] −73.5°, $CHCl_3$), UV λ_{max}^{MeOH} (log ε): end absorption, 230sh (4.15), 270sh (3.16), IR ν_{max}^{KBr} cm^{-1}: 1750, 1710sh, 1630, 1430; ^1H NMR (500 MHz, $CDCl_3$): δ 1.68 (3H, *dd*, *J* = 7.0 and 1.5 Hz, H_3-10), 2.02, 2.03 and 2.04 (12H, each *s*, alcoholic OAc), 2.28 (12H, *s*, phenolic OAc), 2.36 (1H, *dd*, *J* = 14.5 and 9.0 Hz, H-6a), 2.68 (1H, *dd*, *J* = 14.5 and 4.5 Hz, H-6b), 2.90 (2H, *t*, *J* = 7.0 Hz, H_2-2″), 2.96 (2H, *t*, *J* = 7.0 Hz, H_2-2″), 3.76 (1H, *ddd*, *J* = 10.0, 4.5 and 2.5 Hz, H-5′), 3.94 (1H, *dd*, *J* = 9.0 and 4.5 Hz, H-5), 4.10 (1H, *dd*, *J* = 12.0 and 2.5 Hz, H-6′a), 4.18 (1H, *dt*, *J* = 11.0 and 7.0 Hz, H-1″a), 4.26 (1H, *dt*, *J* = 11.0 and 7.0 Hz, H-1″b), 4.30 (1H, *dd*, *J* = 12.0 and 4.5 Hz, H-6′b), 4.32 (1H, *dt*, *J* = 11.0 and 7.0 Hz, H-1″a), 4.36 (1H, *dt*, *J* = 11.0 and 7.0 Hz, H-1″b), 5.02 (1H, *d*, *J* = 8.0 Hz, H-1′), 5.12 (1H, *dd*, *J* = 9.5 and 8.0 Hz, H-2′), 5.12 (1H, *t*, *J* = 9.5 Hz, H-4′), 5.26 (1H, *t*, *J* = 9.5 Hz, H-3′), 5.68 (1H, *qunit like*, *J* = 1.5 Hz, H-1), 5.98 (1H, *br qd*, *J* = 7.0 and ~1.5 Hz, H-8), 7.04 (1H, *d*, *J* = 2.0 Hz, H-4″), 7.06 (1H, *d*, *J* = 2.0 Hz, H-4″), 7.08 (1H, *dd*, *J* = 8.5 and 2.0 Hz, H-8″), 7.10–7.13 (3H, *m*, H_2-7″ and H-8″), 7.42 (1H, *s*, H-3).

Cichoriin (**6**). Mp 210° (ref. [5] 205–220°); $[\alpha]_D^{15}$ −97.60° (50% MeOH; *c* 0.625); UV $\lambda_{max}^{50\%\,dioxane}$ nm (log ε): 227 (4.07), 255 (3.59), 290 (3.69), 345 (3.77); IR ν_{max}^{KBr} cm^{-1}: 3300, 1710sh, 1690, 1680sh, 1570; FAB-MS *m/z* 339 $[M-H]^-$. (Found: C, 52.8; H, 4.8. Calcd. for $C_{15}H_{16}O_9$: C, 53.0; H, 4.7%.) Compound **6** (21.7 mg) was acetylated with pyridine–Ac_2O (each 0.3 ml) in the usual way and the product (23.0 mg) was recrystallized from EtOH to give needles (19.9 mg) of cichoriin pentaacetate (**6a**). Mp 211.5° (ref. [9] 218°), $[\alpha]_D^{20}$ −20.34° ($CHCl_3$; *c* 1.18).

Frachinoside (**4**). $[\alpha]_D^{16}$ −114.10° (MeOH; *c* 0.780); UV λ_{max}^{MeOH} nm (log ε): 227 (4.36), 287 (3.79), 343 (3.86); IR ν_{max}^{KBr} cm^{-1}: 3400, 1720sh, 1710, 1690sh, 1630, 1620sh, 1560, FAB-MS *m/z* 727 $[M+H]^+$. (Found: C, 53.1, H, 5.3. $C_{32}H_{38}O_{19}$ requires: C, 52.9; H, 5.3%.)

Methylation and acid hydrolysis of cichoriin (**6**). A soln of **6** (25.5 mg) in MeOH (8 ml) was treated with excess ethereal CH_2N_2 in the usual way and the product (29.0 mg) hydrolysed with 5% HCl (10 ml) at 95° for 30 min. The reaction mixture was extracted with Et_2O (5 ml × 4) and the dried Et_2O layer was concd *in vacuo*. The residue was purified by prep. TLC ($CHCl_3$–EtOAc, 20:1) to give needles (9.7 mg) of scopoletin (**8**), mp 203° (ref. [10] 203–205°) on recrystallization from MeOH. Identity was confirmed by comparison with an authentic sample [mmp, IR and ^1H NMR]. The aq. layer, on concn *in vacuo*, gave glucose, which was identified with an authentic sample on GC (as TMSi derivatives) (R_t: α, 7.6 min; β, 10.4 min).

Partial hydrolysis of frachinoside (**4**). A soln of **4** (81.8 mg) in 0.2 M NaOH (2.0 ml) was stirred for 6.5 hr at room temp. and neutralized with Amberlite IR-120 B (H^+ form). The residue (86.2 mg), on concn *in vacuo*, was subjected to prep. TLC ($CHCl_3$–MeOH, 7:3) to give cichoriin (**6**) (2.1 mg) and secoxyloganin (**10**) (5.6 mg), $[\alpha]_D^{15}$ −116.67° (MeOH; *c* 1.680, ref. [6] −111.7°, MeOH) [^1H, ^{13}C NMR, see Tables 1 and 2]; 29.4 mg of **4** was recovered.

Acknowledgements—We are grateful to Mr M. Morita (Research Institute for Technology and Science, Kinki University) for mass spectral measurements and to the staff of the Microanalytical Centre of Kyoto University for microanalyses.

REFERENCES

1. Inouye, H., Nishioka, T. and Kaniwa, M. (1975) *Phytochemistry* **14**, 304.
2. LaLonde, R. T., Wong, C. and Tsai, A. I. (1976) *J. Am. Chem. Soc* **98**, 3007
3. Inoue, K., Fujita, T., Inouye, H., Kuwajima, H., Takaishi, K., Tanahashi, T., Nagakura, N., Asaka, Y., Kamikawa, T. and Shingu, T. (1991) *Phytochemistry* **30**, 1191.
4. Kikuchi, M., Yamauchi, Y., Yanase, C. and Nagaoka, I. (1987) *Yakugaku Zasshi* **107**, 245.
5. Steinegger, E. and Brantschen, A. (1959) *Pharm. Acta. Helv.* **34**, 334.
6. Khan, M. A. S., Mooney, E. F. and Stephen, W. I. (1968) *Anal. Chim. Acta.* **43**, 153.
7. Calis, I. and Sticher, O. (1984) *Phytochemistry* **23**, 2539.
8. Kuwajima, H., Uemura, T., Takaishi, K., Inoue, K. and Inouye, H. (1988) *Phytochemistry* **27**, 1757.
9. Head, F. S. H. and Robertson, A. (1939) *J. Chem. Soc.* 1266.
10. Ballantyne, M. M., McCabe, P. H. and Murray, R. D. H. (1971) *Tetrahedron* **27**, 871.