TWO NEW IRIDOIDS FROM *POSOQUERIA LATIFOLIA*

P. D. L. CHAO

China Medical College, Taichung, Taiwan, R.O.C. 400

and

G. H. SVOBODA[1]

ABSTRACT.—From the leaves of *Posoqueria latifolia* two new iridoids, posoquenin **1** and latifonin **4**, were isolated and their structures determined. Structural assignments were based mainly upon the pmr spectra of the parent compounds and their acetates as well as their benzylidene derivatives. The stereochemistry of the compounds was partially determined.

Posoqueria latifolia Roem & Schult (Rubioceae) (1) is also known as *Tocoyena latifolia* Lam. (2) or *Oxyanthus isthmia* Hort. (3). It is one of some 15 species among the genus *Posoqueria* and is indigenous to tropical America and the West Indies (4). Although no medicinal uses have been reported for this genus, the total absence of any phytochemical studies on *Posoqueria* species prompted a phytochemical investigation of this plant. In the course of isolation, two iridoids were encountered and their structural assignments are presented here.

RESULTS AND DISCUSSION

The plant material was extracted by percolation with ethanol to exhaustion. After removal of ethanol, the extract was partitioned between chloroform and water to afford Fraction A (chloroform). The aqueous layer was further partitioned with ethyl acetate to afford Fraction B (ethyl acetate) and Fraction C (water). Chromatography of Fraction A and Fraction B over a silicic acid column yielded posoquenin **1** and latifonin **4**, respectively.

The occurrence of iridoids in Rubiaceous plants is rather common, and they usually are found in the glucoside form (5). The two iridoids described in this paper are not glycosides. Latifonin **4** does not have a double bond between C-3 and C-4 where unsaturation is common in naturally occurring iridoids (6). Several iridoids lacking the typical double bond between C-3 and C-4 have been reported (7) (8) (9) (10).

The first iridoid, posoquenin **1** ($C_{11}H_{14}O_7$), (mass spectrum M^+ at *m/e* 258) was isolated as optically active, colorless crystals. It showed an absorption maximum at 248 nm in the uv spectrum and bands at 3440, 1740, 1688 and 1622 cm^{-1} in the ir spectrum, indicating the presence of a hydroxyl group and two types of carbonyl functions of which one is a saturated ester and the other could be conjugated in an enone-type chromophore. The pmr spectrum of **1** exhibited a singlet at δ1.54 (3H), which was assigned to a tertiary methyl group attached to a carbinol center, and a singlet at δ3.65 (3H) attributable to a carbomethoxy function. A singlet at δ7.31 (1H) is characteristic of the C-3 proton for iridoids which bear a carbonyl function at C-4 (5). Acetylation of **1** (acetic anhydride/pyridine) yielded the diacetate **2** whose pmr spectrum showed two acetyl groups at δ2.04 (3H) and δ2.06 (3H). A singlet appearing at δ5.22 (2H) was assigned to the protons attached to secondary carbinol centers (11). These were seen as doublets at δ3.83 (1H, $J=6$Hz) and δ3.99 (1H, $J=6$Hz) in **1**. The ir spectrum of **2** showed the presence of a hydroxyl function which resisted further attempts

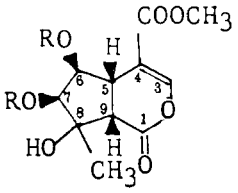
[1]Present Address: 5302 Fallwood Drive, Apt. 105, Indianapolis, IN 46220.

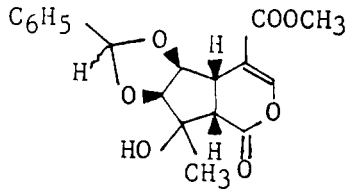
at acetylation. The preceding evidence indicated that the basic skeleton of **1** was methylcyclopentanomonoterpene and the three hydroxyl groups apparently are on the cyclopentane ring.

Concerning the stereochemistry of posoquenin **1**, configurational similarity of **1** with most other iridoids was assumed; the C–5 proton and C–9 proton were thus placed in β-positions (12), although there is one naturally occurring iridoid where the bridge head stereochemistry is trans (8). The configurations around the three carbinol centers need to be determined. The consistently low values of $J5,6(OHz)$ in **1**, **2** and **3** demand a dihedral angle close to 90° between the C–5 proton and C–6 proton, necessitating a trans relationship of these protons (13). As to the position of the hydroxyl group at C–7, the $J6,7$ of **1** and **3** is 6 Hz and is compatible with a cis as well as a trans relationship between the C–6 proton and C–7 proton (13). Other means were, therefore, sought to settle the stereochemistry at this chiral center. Treatment of **1** with benzaldehyde and zinc chloride yielded only one benzylidene derivative **3** which was found rather resistant to acetylation (acetic anhydride/pyridine) even at 80° for 6 hours (14). Prolonged treatment at 80° resulted in decomposition. This fact indicates that the two hydroxyl groups involved in acetal formation are secondary and, therefore, on C–6 and C–7. Consequently, the C–6 proton and C–7 proton are cis to each other and both are in β-positions. Finally, the configuration at C–8 cannot be conclusively determined since no other benzylidene derivatives have been isolated. However, it could be suggested that the hydroxyl group may be in the α-position; otherwise, more than one benzylidene derivative should be obtained in significant amount (14). More positive evidence is necessary for the determination of the configuration of this chiral center. For the comparison with latifonin **4**, reduction of the double bond between C–3 and C–4 was attempted by catalytic hydrogenation with PtO_2 (7) (15) or 10% Pd/C (16) being used as a catalyst and by chemical hydrogenation according to Cortese's method (17) but, unfortunately, the attempt failed. The resistance of this double bond to hydrogenation may be due to two troublesome characteristics—conjugation with a carbonyl function and part of an enol ether. A shortage of starting material precluded further work.

The second iridoid, latifonin **4** ($C_{11}H_{16}O_7$), which differed by H_2 from **1**, was isolated as optically active, colorless crystals. The mass spectrum of **4** showed M^+ at m/e 261; however, elemental analysis indicated no nitrogen content. The peak at m/e 261 was, therefore, considered as a P+1 peak. Further confirmation of the molecular weight of **4** was gained by the mass spectrum of its trimethylsilyl derivative (M^+ at m/e $476=260+72\times3$). The uv spectrum of **4** showed only end absorption; the ir spectrum showed bands at 3480, 3315 and 1720 cm^{-1}, indicating the presence of hydroxyl and ester carbonyl function. The pmr spectrum (DMSO-d_6) exhibited a singlet at δ1.33(3H) assignable to a methyl group attached to a tertiary carbinol center and a singlet at δ3.63(3H) attributable to a carbomethoxy function. The characteristic downfield peak for the C–3 proton of most iridoids was not seen in this compound. Instead, a multiplet between δ4.30 and δ4.50(2H) was seen and can be accounted for by the two non-equivalent protons on C–3. Signals at δ4.67(1H, d, $J=8$Hz), 4.79(1H, d, $J=4$Hz) and 4.98(1H, s) disappeared on the addition of D_2O, which indicated the presence of three hydroxyl groups. Acetylation (acetic anhydride/pyridine) of **4** at room temperature afforded the diacetate **5** whose ir spectrum showed the presence of an unreacted hydroxyl function (3400 cm^{-1}) which resisted further attempts at acetylation. The pmr spectrum of **5** exhibited signals for two acetyl groups at

δ1.97(3H, s) and δ2.09(3H, s). The signals at δ5.07(1H, d, $J=4$Hz) and 5.55(1H, q, $J=4$Hz) shifted downfield for 1.64δ and 1.41δ, respectively, when **4** was converted into **5**. This shift indicated that there are two secondary alcoholic functions vicinal to each other (11). The preceding evidence suggested that the skeleton and also the hydroxylation pattern of **4** were similar to that of **1**. The

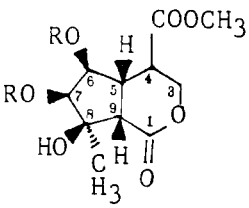




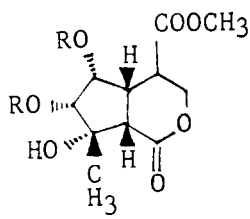
1 R = H

2 R = Ac

3

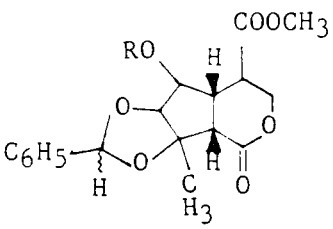


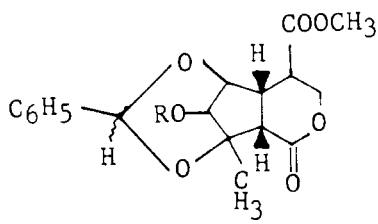
OR



4 R = H

5 R = Ac





6 R = H

8 R = Ac

7 R = H

9 R = Ac

similarity of the pmr spectra between **1** and **4** can be seen in table 1. As to the assignments of the stereochemistry around the six chiral centers, the C–5 and C–9 protons were first assumed to be in the β-positions, as in posoquenin (12). The coupling constants $J5,6$ and $J6,7$ are 4Hz, which is compatible with cis as well as

trans relationships (12). Benzylidene derivatives were, therefore, prepared in order to determine the relative configuration of the three hydroxyl functions. Two benzylidene derivatives, **6** and **7**, were isolated from the reaction mixture and their structural assignments were further confirmed by acetylation (acetic anhydride/pyridine), which afforded **8** and **9**, respectively. These results indicated that all three hydroxyl groups are on the same side of the cyclopentane ring. However, whether they are in α- or β-positions cannot be determined here.

TABLE 1. Pmr data of compound **1–9** (60MHz, int. ref. Me_4Si; J in Hz).

	Solvent	3-H	6-H	7-H	8-CH_3	$COOCH_3$
1	CD_3OD	7.31,s	3.99,d,J=6	3.83,d,J=6	1.54,s	3.65,s
2	$CDCl_3$	7.31,s	5.22,s	5.22,s	1.54,s	3.65,s
3	$CDCl_3$	7.33,s	4.43,d,J=6	4.13,d,J=6	1.66,s	3.66,s
4	CD_3OD	4.45-, m*	4.27,q,J=4	3.56,d,J=4	1.48,s	3.72,s
4	DMSO-d_6	4.30–4.50,m	3.93–4.30,m	3.41,q,J=4	1.33,s	3.63,s
5	$CDCl_3$	4.37–4.64,m	5.55,q,J=4	5.07,d,J=4	1.50,s	3.67,s
6	CD_3OD	4.30–, m*	4.57,q,J=4	5.22,d,J=4	1.44,s	3.74,s
7	CD_3OD	4.46–, m*	5.48,q,J=4	4.00,d,J=4	1.53,s	3.67,s
8	$CDCl_3$	4.47–4.73,m	5.59,q,J=4	5.22,d,J=4	1.62,s	3.71,s
9	$CDCl_3$	4.33–4.73,m	5.72,q,J=4	5.26,d,J=4	1.53,s	3.61,s

*Partially overlapped with solvent peak.

EXPERIMENTAL[2]

PLANT MATERIAL.—The leaves of *P. latifolia* (Rubiaceae) were used. The plant was identified by F. J. Simmonds, and a voucher specimen is on deposit at Eli Lilly and Co., Indianapolis, IN 46206.

EXTRACTION AND FRACTIONATION.—Leaves of *Posoqueria latifolia* (Rubiaceae) (3.3 kg) were percolated with ethanol to exhaustion. After evaporation of the ethanol, the residue was dissolved in water (2 liters) and the aqueous fraction was partitioned with chloroform (2 x 2 liters) to afford Fraction A (chloroform, 7.4 gm). The aqueous fraction was further partitioned with ethyl acetate (2 x 2 liters) to afford Fraction B (ethyl acetate, 29.0 gm) and Fraction C (water, 200 gm).

ISOLATION OF POSOQUENIN **1**.—Fraction A was chromatographed on a silicic acid column (5 cm x 60 cm) and eluted with solvent in a discontinuous gradient going from petroleum ether to benzene to chloroform. The fraction eluted with chloroform afforded posoquenin **1** (650 mg) which was recrystallized several times from ethyl acetate. It gave mp 150–151°; $[\alpha]^{25}D$ −7.4° (c 1.0, MeOH); uv: λ max (MeOH) 248 nm (log ε 4.04); ir: ν max (KBr) 3440, 1740, 1688 and 1622 cm^{-1}; pmr (CD_3OD): δ 1.54 (3H,s) 3.02 (1H, d, J=4Hz), 3.31 (1H,d, J=4Hz), 3.65 (3H,s), 3.83 (1H,d, J=6Hz), 3.99 (1H,d, J=6Hz), 7.31 (1H,s); ms: m/e 258 (M^+ for $C_{11}H_{14}O_7$; 19%), 240 (2), 227(7), 226(6) and 153(100).

ACETYLATION OF **1**.—Compound **1** (32 mg) was treated overnight with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature. After the usual work-up, an oily liquid was obtained and then purified by preparative tlc (silica gel G; ethyl acetate:chloroform=1:3). Crystallization of this oil has not been accomplished. The following data were obtained: pmr ($CDCl_3$): δ 1.56 (3H,s), 2.04 (3H,s), 3.12 (1H,d, J=4Hz), 3.47 (1H,d, J=4Hz), 3.65 (3H,s), 5.22 (2H,s), 7.37 (1H,s).

[2]All mps were taken on a Thomas-Hoover Unimelt Capillary Apparatus or a Yanaco Micro Melting Point Apparatus and are uncorrected. Ir spectra were determined in KBr discs on a Perkin-Elmer model 267 Spectrometer or a Shemadru IR-400 Spectrometer. Optical rotations were determined in methanol solutions on a Perkin-Elmer model 202 Spectrometer. Pmr spectra (60 MHz) were recorded on a JNM-PMX 60 PMR Spectrometer or on a Hitachi Perkin-Elmer R-24 instrument; chemical shifts are reported in δ values. Routine mass spectra were determined on an LKB-9000 Spectrometer, by either direct probe or glc:ms technique. In the latter case, the glc column (2 x 0.004 m, glass) was packed with 3% OV-17 on 80–100 mesh Supelcorport (Supelco Inc., Bellefonte, Pa.). Following sample injection, the temperature of the glc oven was held at 130° for 1 min., then raised by 10°/min. to 270° where it was held for 30 min.

PREPARATION OF BENZYLIDENE DERIVATIVE OF **1**.—Compound **1** (200 mg) was stirred with *freshly distilled benzaldehyde* (10 ml) and *anhydrous zinc chloride* (0.5 gm) for 3.5 hrs at room temperature (6). The reaction mixture was poured into saturated sodium bicarbonate solution (40 ml) and extracted with *n*-hexane (3 x 40 ml), which was then discarded. The aqueous solution was extracted with ethyl acetate (3 x 60 ml) which, after drying, was concentrated *in vacuo*. The residue obtained was subjected to column chromatography (silicic acid, 1.5 cm x 20 cm) and eluted with chloroform to yield **3** (90 mg). It gave the following data: mp 166–168°; ir: ν max (KBr) 3100, 1740, 1665 and 1615 cm^{-1}; uv: λ max (MeOH) 241 nm (log ϵ 3.78); pmr ($CDCl_3$): δ 1.66 (3H,s), 3.20 (1H,d, J=4Hz), 3.56 (1H,d, J=4Hz), 3.66 (3H,s), 4.13 (1H,d, J=6Hz), 4.43 (1H,d, J=6Hz), 5.53 (1H,s) and 7.33 (6H, br.s); ms: *m/e* 346 (M^+ for $C_{15}H_{18}O_7$, 10%), 315(17), 241(12), 240(77), 222(31), 207(39) and 197(100).

ATTEMPTED ACETYLATION OF **3**.—Compound **3** (72 mg) was treated overnight with acetic anhydride (3 ml) and pyridine (3 ml) at room temperature. After the usual work-up, **3** was recovered unchanged. Acetylation was then attempted at 80° with stirring for 6 hrs. Again, the starting material was obtained. Prolonged treatment at 80° for 30 hrs resulted in decomposition.

ISOLATION OF LATIFONIN **4**.—Fraction B was chromatographed on a silicic acid column (7 cm x 70 cm) and eluted in a discontinuous gradient going from petroleum ether to benzene to chloroform to methanol. The fractions eluted with chloroform-methanol (92:8) afforded latifonin **4** (810 mg), which was recrystallized from ethyl acetate to give mp 157–159°; $[\alpha]^{25}D$ +7.2° (c 1.2, MeOH); uv; λ max (MeOH) 210 nm (log ϵ 2.26); ir: ν max (KBr) 3480, 3315 and 1720 cm^{-1}; pmr (DMSO-d_6): δ 1.33 (3H,s), 2.60–3.00 (3H,m), 3.41 (1H,q, J=4Hz), 3.63 (3H,s), 3.93–4.30 (1H,m), 4.30–4.50 (2H,m), 4.67 (1H,d, J=8Hz), 4.79 (1H,d, J=4Hz) and 4.98 (1H,s); ms: *m/e* 261(p+1, 0.5%), 242(2), 224(9) and 199(100). Elemental analysis: Found: C, 50.32; H, 6.32%, ($C_{11}H_{16}O_7$ requires: C, 50.76; H, 6.15%).

ACETYLATION OF **4**.—Compound **4** (28 mg) was treated overnight with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature. After work-up, 18 mg of crystals of **5** were obtained. These were recrystallized from a petroleum ether-benzene mixture to give mp 141–143°; ir: ν max (KBr) 3400, 1753, 1733 and 1712 cm^{-1}; pmr ($CDCl_3$): δ 1.50 (3H,s), 1.97 (3H,s), 2.09 (3H,s), 2.70–3.80 (3H,m), 3.67 (3H,s), 4.37–4.64 (2H,m), 5.07 (1H,d, J=4Hz) and 5.55 (1H,q, J=4Hz); ms: *m/e* 345 (p+1, 0.5%), 242(19), 241(100) and 236(36).

PREPARATION OF BENZYLIDENE DERIVATIVES OF **4**.—Compound **4** (700 mg) was stirred with freshly distilled benzaldehyde (23 ml) and anhydrous zinc chloride (1.2 g) for 3 hrs at room temperature (9). The reaction mixture was poured into saturated sodium bicarbonate solution (100 ml) and extracted with *n*-hexane (3 x 100 ml), which was then discarded. The aqueous solution was extracted with ethyl acetate (3 x 150 ml) which, after drying, was concentrated *in vacuo*. The residue was chromatographed over a silicic acid column (3 cm x 50 cm) and eluted with chloroform-ethyl acetate (1:1). The faster running compound was recrystallized from petroleum ether-ethyl acetate mixture to yield **6** (82 mg) mp 177–179°; ir: ν max (KBr) 3450, 1720, 1590, 1575 and 1440 cm^{-1}; pmr (CD_3OD): δ 1.44 (3H,s), 2.83–3.17 (3H,m), 3.74 (3H,s), 4.57 (1H,q, J=4Hz), 5.22 (1H,d, J=4Hz), 7.40–7.67 (3H,m) and 7.93–8.20 (2H,m); ms: *m/e* 366 (for $C_{15}H_{20}O_7 \cdot H_2O$, 0.5%), 365(2), 348(1), 333(4), 303(33), 242(16), 224(60), 99(100). The slower running compound eluted as a mixture with **5**. Further separation was achieved by rechromatography on another silicic acid column (1.5 cm x 20 cm). Elution with chloroform: ethyl acetate (1:1) yielded **7** (31 mg) as an oil which resisted all attempts at crystallization. The compound showed pmr (CD_3OD): δ 1.53 (3H,s), 2.83–3.13 (3H,m) 3.67 (3H,s), 4.00 (1H,d, J=4Hz), 5.48 (1H,q, J=4Hz), 7.23–7.67 (3H,m) and 7.93–8.20 (2H,m).

ACETYLATION OF **6**.—Compound **6** (40 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) overnight at room temperature. After the usual work-up, an oil (38 mg) was obtained: attempts at crystallization failed. Further purification done on preparative tlc plates (silica gel G; ethyl acetate:chloroform=1:1) afforded a homogeneous **8**. The compound showed ir: ν max ($CHCl_3$) 3400, 1715, 1600, 1585, 1460 and 1440 cm^{-1}; pmr ($CDCl_3$): δ 1.62 (3H,s), 1.93 (3H,s), 2.73–3.40 (3H,m), 3.71 (3H,s), 4.47–4.73 (2H,m), 5.22 (1H,d, J=4Hz), 5.59 (1H,q, J=4Hz), 7.33–7.60 (3H,m) and 7.80–8.07 (2H,m).

ACETYLATION OF **7**.—Compound **7** (31 mg) was treated overnight at room temperature with acetic anhydride (1 ml) and pyridine (1 ml). After work-up, the mixture, when subjected to further purification on preparative tlc plates (silica gel G; ethyl acetate-chloroform=1:1), yielded **9** (25 mg) mp 204–205°; ir: ν max (KBr) 3440, 1730, 1720, 1600, and 1580 cm^{-1}; pmr ($CDCl_3$): δ 1.53 (3H,s), 2.07 (3H,s), 2.83–3.47 (3H,s), 3.61 (3H,s), 4.33–4.73 (2H,m), 5.26 (1H,d, J=4Hz), 5.72 (1H, q, J=4Hz), 7.27–7.60 (3H,m) and 7.73–8.03 (2H,m); ms: *m/e* 408 (for $C_{20}H_{22}O_8 \cdot H_2O$, 0.2%), 407(0.5), 375(3), 315(5) and 303(50).

ACKNOWLEDGMENTS

The mass spectrometer facility was supported by Research Grant 5S01 RR05455-10 from the National Institutes of Health. The authors are grateful to Mr. John Naworal, Graduate

School of Public Health, University of Pittsburgh, for determining the mass spectra and to Analytical Center, Kyoto University, for the elemental analysis.

Received 22 October 1979.

LITERATURE CITED

1. B. D. Jackson, "Index Kewensis" vol. II, University Press, Oxford, England, (1895) p. 608.
2. ibid., p. 1086.
3. "Index Kewensis" Sixth supplement, 1916–1920, University Press, Oxford, England, (1926), p. 334.
4. G. H. M. Lawrence "Taxonomy of Vascular Plants", Mac Millan Co., New York (1951), p. 712–713.
5. Y. Takeda, H. Nishimura and H. Inouye, *Phytochemistry*, **16**, 1401 (1977).
6. V. Plouvier and J. Favre-Bonvin, *Phytochemistry*, **10**, 1697 (1971).
7. H. Taguchi, Y. Yokokawa and T. Endo, *Yakugaku Zasshi*, **93**, 607 (1973).
8. S. M. Kupchan, A. L. Dessertine, B. T. Blaylock and R. F. Bryan, *J. Org. Chem.*, **39**, 2477 (1974).
9. C. R. Hutchinson, M. Nakane, D. VanEngen and J. Clardy, *J. Am. Chem. Soc.*, **100**, 7079 (1978).
10. R. K. Chaudhuri and O. Sticher, *Helv. Chim. Acta*, **62**, 644 (1979).
11. N. B. Bhacca and D. H. William, "Application of NMR Spectroscopy in Organic Chemistry", Holden-day, San Francisco, (1964), p. 77.
12. J. M. Bobbitt, K. P. Segebarth, W. I. Taylor and A. R. Battersby, Eds., "Cyclopentanoid Terpene Derivatives", Dekker, New York, (1969), p. 1.
13. M. Booth, *Progr. Nucl. Magn. Resonance Spectrosc.*, **5**, 149 (1970).
14. P. Eigtved, S. R. Jensen and B. J. Nielsen, *Acta. Chem. Scand.* **B28**, 87 (1974).
15. H. Inouye, T. Yoshida, S. Tobita and M. Okigawa, *Tetrahedron*, **26**, 3905 (1970).
16. C. H. Brieskorn and R. Ahlborn, *Tetrahedron Letters*, **41**, 4037 (1975).
17. N. A. Cortese and R. F. Heck, *J. Org. Chem.*, **20**, 3985 (1978).