Journal of Natural Products
Vol. 48, No. 1, pp. 108-110, Jan-Feb 1985

PERICLYMENOSIDIC ACID, A NEW BIOSIDIC ESTER IRIDOID GLUCOSIDE FROM *LONICERA COERULEA*

IHSAN CALIS[1] and OTTO STICHER

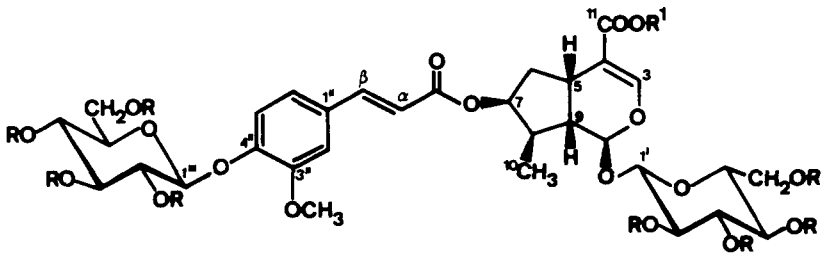
School of Pharmacy, Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

As a part of systematic isolation and structure determination of cyclopentanoid monoterpene glucosides from various plants belonging to the family *Caprifoliaceae*, the water-soluble constituents of leaves and stem bark from *Lonicera coerulea* L. were investigated. In addition to several known iridoids (loganin, loganic acid) and secoiridoids (secologanin, secologanic acid, sweroside), a new biosidic ester iridoid, named periclymenosidic acid (**1**), has been isolated. The structure of **1** was determined by spectral (^{1}H and ^{13}C nmr) and chemical evidence.

RESULTS AND DISCUSSION

Periclymenosidic acid (**1**), $[\alpha]^{20}D = -54.7°$ (c=0.56 in MeOH), was obtained as an amorphous powder. The molecular formula $C_{32}H_{42}O_{18}$ is based on fast atomic bombardment mass spectrometry, chemical correlation of **1** with **2**, the number of signals in the PND ^{13}C nmr and their multiplicities in the SFORD and integration of the ^{1}H-nmr spectra of **1** and its peracetate **3**.

Acid hydrolysis of **1** in refluxing aqueous 3N HCl yielded glucose and ferulic acid. ^{1}H- and ^{13}C-nmr spectra of **1** confirmed the iridiod structure and showed the presence of two sugar and one acyl moieties. The ^{1}H-nmr spectrum showed the three aromatic protons of the ferulic residue as two broad singlets at 7.17 ppm (H-5″, H-6″) and 7.27 ppm (H-2″). This deceptively simple spectrum is, compared with the ABX spectrum of unsubstituted ferulic acid, due to the glycosylation at OH-4″ (*the glycosylation at C-4″ is also verified* through ^{13}C-nmr chemical shift differences compared with model compounds [cf., (1) and references therein]. The AX system belonging to the olefinic protons of the ferulic acid at δ 6.45 (d, *J* 15.9 Hz, H-α) and 7.63 (d, *J* 15.9 Hz, H-β) indicated a *trans*-configuration of the olefinic double bond. Furthermore, close similarities between ^{1}H- and ^{13}C-nmr spectra of **1** and periclymenoside (**2**) (1)[2] were observed, the only difference lying in the $COOCH_3$ signals. Compared with the nmr spectra of peric-



1 R=H, R^1=H
2 R=H, R^1=CH_3
3 R=Ac, R^1=H

[1]Research Associate at School of Pharmacy, ETH Zurich, during January 1982, until December, 1983, from Faculty of Pharmacy. Department of Pharmacognosy, Hacettepe University, Ankara, Turkey.

[2]Inadvertently, we have drawn in the paper of reference (1) the structure of the glucose moiety attached at C-4″ in periclymenoside as α-L-glucose; it should be β-D-glucose.

lymenoside, in **1** a three proton singlet at ~3.7 ppm in the ^1H nmr and a quartet at ~52 ppm in the ^{13}C nmr are absent (Table 1). These results and the fact that **1** after esterification with CH_2N_2 afforded **2** led us to conclude that **1** is the demethyl derivative of **2**. The acyloxy unit is substituted at C-7 as in **2** (1). This is based on the expected downfield shift ($\Delta\delta = +3.75$ ppm compared with loganic acid) for the signal of the carbon atom in α-position (C-7) and the high-field shifts ($\Delta\delta = -2.00$ ppm and -1.84 ppm) for the signals of the carbon atoms in β-positions (C-6 and C-8) [cf., (2)].

Based on these data, periclymenosidic acid was identified as 7-*O*-[4″-*O*-(β-D-glucopyranosyl)-*trans*-feruloyl] loganic acid (=demethyl-periclymenoside).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The general experimental procedures were as reported in a recent paper (1). Fab ms was recorded with a Kratos MS 50 FAB-System (M-Scan Ltd) spectrometer. For droplet countercurrent chromatography (dccc), Model DCC-A (Tokyo Rikakikai, Toyko, Japan) equipped with 300 standard glass tubes (40 cm×2.0 mm i.d.) was used.

PLANT MATERIAL.—The plant material (leaves and stem bark) was collected near St. Moritz in Switzerland in August 1982. A voucher specimen is deposited in the Herbarium of the Laboratory of Pharmacognosy and Phytochemistry, School of Pharmacy, ETH Zurich.

EXTRACTION AND SEPARATION OF IRIDOIDS AND SECOIRIDOIDS FROM LEAVES.—Fresh leaves of *L. coerulea* (1 kg) were extracted as reported before (1). Forty grams of the aqueous extract (84 g) was dissolved in H_2O and filtered through an aluminium oxide column (200 g) to yield 23 g extract. Chromatography of this pre-purified extract over silica gel (500 g) and elution with increasing gradients of CH_2Cl_2-MeOH-H_2O (80:20:2, 70:30:3 and 60:40:4) afforded ten fractions (fr. 1-10).

Fraction 3 (560 mg) afforded secologanin, which was purified by semipreparative hplc (MeOH-H_2O, 35:65; flow rate 10 ml/min). It was identified by spectral data and by comparison with an authentic sample.

Fraction 4 (2.3 g) yielded sweroside and loganin which were purified by dccc ($CHCl_3$-MeOH-H_2O, 43:37:20; lower phase was used as mobile phase). Their spectra and physical data were in accordance with those reported earlier (1,3).

Fraction 7 (577 mg) was rechromatographed on a silica gel column using EtOAc-MeOH-PrOH-H_2O (80:15:5:5) as eluent to yield secologanic acid. The spectra of secologanic acid and its derivatives which were obtainedd by acetylation and esterification were in good agreement with the reported data (4,5).

Fraction 9 (1.5 g), which showed mainly two

TABLE 1. ^{13}C-nmr (75.47 MHz) spectral data[a]

C-Atom	1	2 (1)	C-Atom	1	2 (1)
1	97.73 d	97.79 d	1″	130.56 s	130.53 s
3	152.51 d	152.80 d	2″	112.50 d	112.55 d
4	113.62 s	113.17 s	3″	151.01 s	150.97 s
5	32.98 s	32.81 d	4″	150.09 s	150.08 s
6	40.59 t	40.55 t	5″	117.41 d	117.41 d
7	78.75 d	78.71 d	6″	123.67 d	123.74 d
8	41.19 d	41.15 d	α	117.63 d	117.60 d
9	47.21 d	47.19 d	β	146.10 d	146.20 d
10	14.01 q	14.02 q	Ar-OCH_3	56.91 q	57.00 q
11	171.10 s	169.47 s	C=O	168.59 s	168.61 s
$COOCH_3$		52.04 q			
1′	100.27 d	100.31 d	1‴	102.23 d	102.24 d
2′	74.83 d	74.82 d	2‴	74.83 d	74.82 d
3′	78.06 d	78.03 d	3‴	77.86 d	77.82 d
4′	71.68 d	71.65 d	4‴	71.33 d	71.33 d
5′	78.39 d	78.38 d	5‴	78.29 d	78.27 d
6′	62.88 t	62.88 t	6‴	62.55 t	62.58 t

[a]The spectrum was recorded in CD_3OD. Chemical shifts are in ppm relative to internal TMS.

polar iridoids besides a high amount of mono- and di-saccharides, was treated with charcoal (25 g) and stratified on a layer of celite in a gooch funnel. The mono- and di-saccharides were removed by washing the funnel first with H_2O then with 10% MeOH until no sugar could be detected in the filtrate. The iridoids were obtained after subsequent treatment with MeOH. The filtrate was evaporated in vacuo and the residue was chromatographed over polyamide (150 g). Elution with H_2O gave secologanic acid and finally loganic acid which was identified by spectral data and comparison with an authentic sample (1).

EXTRACTION AND SEPARATION OF IRIDOIDS FROM THE STEM BARK.—Stem bark (250 g) was extracted as previously reported (1) to yield 18 g crude extract. The crude extract was chromatographed on silica gel (300 g) with *n*-BuOH-EtOH-H_2O (10:2:2) to yield in fraction *a* (790 mg), secologanin and sweroside; in fraction *b* (1.115 g), mainly loganin; in fraction *c* (2.38 g), loganin and secologanic acid, and finally in fraction *d* (2.2 g), periclymenosidic acid (**1**) besides loganic acid and mono- and di-saccharides.

ISOLATION OF PERICLYMENOSIDIC ACID (**1**).—Fraction *d* (1 g) was chromatographed on polyamide (35 g). Elution with H_2O gave loganic acid as well as mono- and di-saccharides. The following elution with MeOH gave mainly periclymenosidic acid (120 mg) which was purified by semipreparative hplc (MeOH-H_2O, 40:60; flow rate, 10 ml/min). $[\alpha]^{20}D = -54.7°$ (c=0.56 in MeOH); uv λ max (MeOH) 217 (log ε 4.21), 230 (log ε 4.18), 293 (log ε 4.07), and 317 nm (log ε 4.08); ir λ max (KBr) 3420, 1690, 1630, and 1505 cm^{-1}; fab ms *m/z* 737 $[M+Na]^+$, 715 $[M+H]^+$ (calcd for $C_{32}H_{42}O_{18}$, 714,682); ^1H nmr (300.13 MHz, MeOH-d_4) δ 1.11 (3H, d, *J* 6.7 Hz, H-10), 1.82 (1H, m, αH-6), 2.14 (1H, m, βH-6), 2.17 (1H, m, H-8), 2.36 (1H, dd, *J* 14.5/7.8 Hz, H-9), 3.16 (1H, m, H-5), 3.20-3.55 (8H, m, H-2′, H-3′, H-4′, H-5′, H-2‴, H-3‴, H-4‴, H-5‴), 3.90 (3H, s, Ar-OCH_3), 3.87-3.93 (2H, m, H-6′a, H-6‴a), 3.67-3.76 (2H, m, H-6′b, H-6‴b), 4.69 (1H, d, *J* 7.8 Hz, H-1′), 4.97 (1H, d, *J* 7.2 Hz, H-1‴), 5.27-5.31 (2H, m, H-1, H-7), 6.45 (1H, d, *J* 15.9 Hz, H-α), 7.17 (2H, br.s., H-5″, H-6″), 7.27 (1H, br.s., H-2″), 7.43 (1H, s, H-3), 7.63 (1H, d, *J* 15.9 Hz, H-β); ^{13}C nmr (75.47 MHz, MeOH-d_4) see Table 1.

ACETYLATION OF **1**.—Acetylation of **1** (10 mg) with Ac_2O/pyridine at room temperature for 24 h followed by chromatography on silica gel with Et_2O-EtOAc (1:1) gave the octaacetate **3**. ^1H nmr (300.13 MHz, $CHCl_3$-d) δ 1.08 (3H, d, *J* 6.7 Hz, H-10), 1.87-2.09 (2H, m, αH-6, βH-6), 2.28-2.37 (2H, m, H-8, H-9), 3.07 (1H, m, H-5), 3.73-3.87 (2H, m, H-5′, H-5‴), 3.87 (3H, s, Ar-OCH_3), 4.16 (2H, m, H-6′a, H-6‴a), 4.30 (2H, m, H-6′b, H-6‴b), 4.87 (1H, d, *J* 8.1 Hz, H-1′), 5.22 (1H, d, *J* 9.4 Hz, H-1‴), 4.97-5.33 (7H, m, H-1, H-2′, H-3′, H-4′, H-2‴, H-3‴, H-4‴), 6.30 (1H, d, *J* 16 Hz, H-α), 7.05-7.12 (3H, m, H-2″, H-5″, H-6″), 7.41 (1H, d, *J* 0.9 Hz, H-3), 7.57 (1H, d, *J* 16 Hz, H-β). Additional signals: 2.09, 2.07 (2x), 2.04, 2.033, 2.029, 2.00, 1.95 (each 3H, s, 8x $OCOCH_3$).

ESTERIFICATION OF **1**.—Periclymenosidic acid (10 mg) was esterified with CH_2N_2 in the usual way to yield periclymenoside (**2**). ^1H-nmr data of **2** were in good agreement with those reported (1). For ^{13}C nmr, see Table 1.

ACID HYDROLYSIS OF **1**.—Acid hydrolysis and detection of ferulic acid and glucose were made as reported before (1).

ACKNOWLEDGMENTS

This work was supported by a research grant of the Swiss National Science Foundation. Thanks are due to Daniel Lehmann for technical help; Dr. J. Meili, Institute of Organic Chemistry, ETH Zurich, for the fab ms; and Mrs. J. Schmid-Kyzintas for secretarial help.

LITERATURE CITED

1. I. Calis, M.F. Lahloub, O. Sticher, *Helv. Chim. Acta*, **67**, 160 (1984).
2. R.K. Chaudhuri, F.Ü. Afifi-Yazar, O. Sticher, and T. Winkler, *Tetrahedron*, **36**, 2317 (1980).
3. T.A. van Beek, P.P. Lankhorst, R. Verpoorte, and A. Baerheim Svendsen, *Planta Med.*, **44**, 30 (1982).
4. J.P. Chapelle, *Planta Med.*, **29**, 268 (1976).
5. A. Cornelis and J.P. Chapelle, *Pharm. Acta Helv.*, **51**, 177 (1976).

Received 23 January 1984