Journal of Natural Products
Vol. 52, No. 1, pp. 135-142, Jan-Feb 1989

LACINIATOSIDE V: A NEW BIS-IRIDOID GLUCOSIDE. ISOLATION AND STRUCTURE ELUCIDATION BY 2D NMR SPECTROSCOPY

BENJAMIN PODÁNYI,[1,*] R. STEPHEN REID,

Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

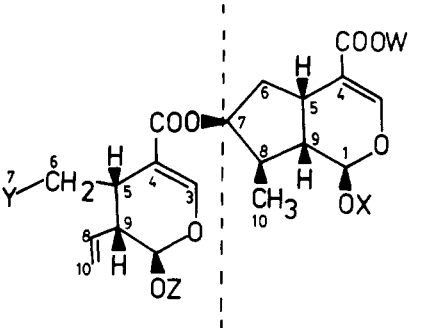
AKOS KOCSIS, and LÁSZLÓ SZABÓ

Institute of Organic Chemistry of the Semmelweis Medical University, H-1092 Budapest, Högyes E. u.7, Hungary

ABSTRACT.—A new bis-iridoid glucoside, laciniatoside V [**1**], has been isolated from *Dipsacus laciniatus* and its structure elucidated by nmr spectroscopy. Complete analysis and unambiguous assignment of the ^{1}H- and ^{13}C-nmr spectra were achieved by 2D shift correlation measurements and spectral simulation, the first time that this has been accomplished for a bis-iridoid glucoside. The vicinal spin-spin coupling constants of the ^{1}H-nmr spectrum have also been used to characterize the conformation of the iridoid rings.

Iridoids represent a large group of cyclopentano[*c*]pyran monoterpenoids (1). The structure of secoiridoids is characterized by a cyclopentano[*c*]pyran system cleaved between C-7 and C-8. They are found as natural products in a large number of plant families, usually as glucosides. The first bis-iridoid compound, cantleyoside, containing a secoiridoid unit and an iridoid unit, was found in *Cantleya corniculata* (Icacinaceae) (2) and later isolated also from a *Dipsacus* species (3). Jensen *et al.* (4) isolated four new bis-iridoids from *Dipsacus sylvestris* and named them sylvestrosides I–IV. Figure 1 shows some representative structures. Recently, we isolated five new bis-iridoid glucosides from *Dipsacus laciniatus* L. (Dipsacaceae), and named them laciniatosides I–V (5,6).

Only a few characteristic signals have previously been reported from the ^{1}H-nmr spectra of the bis-iridoids (4–6), because of severe overlapping of the peaks at lower field strengths. We therefore decided to employ 2D nmr techniques at higher field to assign completely the ^{1}H-nmr spectra of these compounds. Heteronuclear ^{13}C/^{1}H shift correlation experiments also make possible unambiguous assignment of the ^{13}C-nmr spectra



Secoiridoid unit | Iridoid unit

	W	X	Y	Z
Sylvestroside I	Me	Glc	$-CH_2OH$	Glc
Sylvestroside II	Me	Glc	$-CH_2OAc$	Glc
Sylvestroside III	Me	H	$-CHO$	Glc
Cantleyoside	Me	Glc	$-CHO$	Glc

FIGURE 1. The structure of some bis-iridoids. Glc = β-D-glucose.

[1]Present address: Chinoin Pharmaceutical and Chemical Works Ltd., H-1045 Budapest, Tó u. 1-5, Hungary.

of the bis-iridoids. (Previous assignments are uncertain to some extent, because they are based upon comparison with model compounds and upon chemical modification.) Here we report the isolation and the structure elucidation of laciniatoside V [**1**], through the complete assignment of its ^{1}H- and ^{13}C-nmr spectra using these techniques.

RESULTS AND DISCUSSION

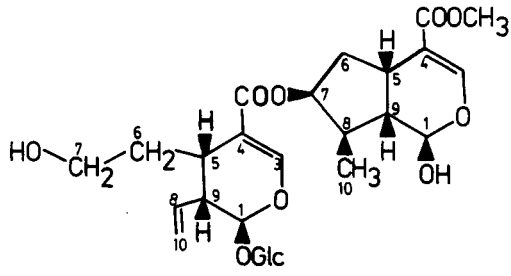
In the isolation of laciniatoside V [**1**], the glycoside fraction was extracted from leaves and stalks of the flowering *D. laciniatus*. Laciniatoside V was then isolated from this fraction by countercurrent distribution and repeated cc.

The one-dimensional (1D) ^{1}H- and ^{13}C-nmr spectra of the compound in acetone-d_6 displayed signals which, on the basis of previous work (5,6), indicated the presence of one iridoid, one secoiridoid, and one glucose unit. A ^{1}H singlet at 3.67 ppm and a ^{13}C resonance at 51.3 ppm (which appears with positive phase in the gated-decoupled spin-echo spectrum) suggested strongly that the carboxylate function of either the iridoid or the secoiridoid unit is in the methyl ester form. In the ^{1}H-nmr spectrum, adding D_2O to the solution caused the disappearance of the doublet at 6.40 ppm and the collapse of the pseudo-triplet at 5.00 ppm into a doublet. This indicated that there is a free hydroxy group on the iridoid or the secoiridoid unit.

The COSY-90 spectrum made it possible to separate the ^{1}H-nmr spectral signals into three sets (the iridoid, secoiridoid, and glucose units). The ^{1}H signals at 7.42 ppm and 7.52 ppm have chemical shifts characteristic of the C-3 protons in iridoid and seco-iridoid structures, respectively (4). By following the pathway of the off-diagonal peaks in the COSY-90 plot, the entire spin-spin coupling networks of the iridoid and seco-iridoid skeletons could then be elucidated, and the signals assigned. The chemical shifts and the coupling constants of these two sets of signals correspond to a loganin moiety with a free hydroxy group at C-1 and a secologanin-glucoside with a reduced aldehyde group. The high ^{1}H chemical shift (5.24 ppm) of the proton signal of the loganin moiety indicated that this group is esterified with the carboxyl group of the secologanin unit.

This completed the structure of laciniatoside V [**1**] shown in Figure 2. The present compound is closely related to the previously isolated bis-iridoids of the *Dipsacus* species; it is the loganin-aglucone analogue of sylvestroside I. Similarly, sylvestroside III is the loganin-aglucone analogue of cantleyoside.

A set of proton signals with about 10% the intensity of those assigned above could also be observed in the spectra. These are probably due to the isomer with a 1α-hydroxy group in the loganin unit. This isomer may be in equilibrium with laciniatoside V, which has a 1β-hydroxy group. Such an effect has been previously observed; during the



A B

1

FIGURE 2. The structure of laciniatoside V [**1**], Glc = β-D-glucose. A, the secologanin unit; B, the loganin-aglucone unit.

formation of loganin-aglucone from loganin, the *epi*-1-hydroxy isomer was also detected (7).

Approximate ^1H chemical shifts and coupling constants can be determined by first-order spectral analysis of the 1D ^1H spectrum, after Gaussian resolution enhancement. The C-8 proton of the loganin unit gives rise to a complicated multiplet, so to determine coupling constants to this proton, homodecoupling was applied at the C-10 methyl proton frequency. Couplings to the C-7 and C-9 protons were then readily determined by inspection of the resultant doublet of doublets.

These approximate chemical shifts and coupling constants were then used as initial estimates for second-order analysis. The parameters were varied until agreement between measured and calculated spectra was optimal, as shown in Figures 3 and 4. Spectral parameters are listed in Table 1.

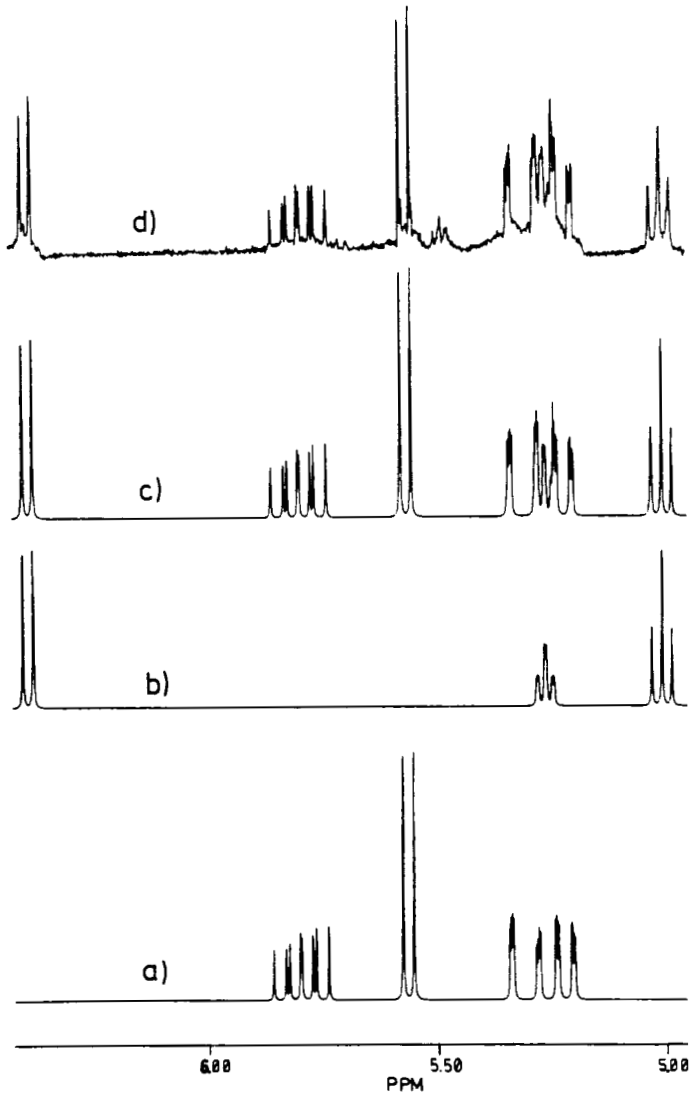


FIGURE 3. The high-frequency part of the spectral simulation of laciniatoside V [**1**]. a, simulation, secologanin unit; b, simulation, loganin unit; c, superposition of a and b; d, measured spectrum.

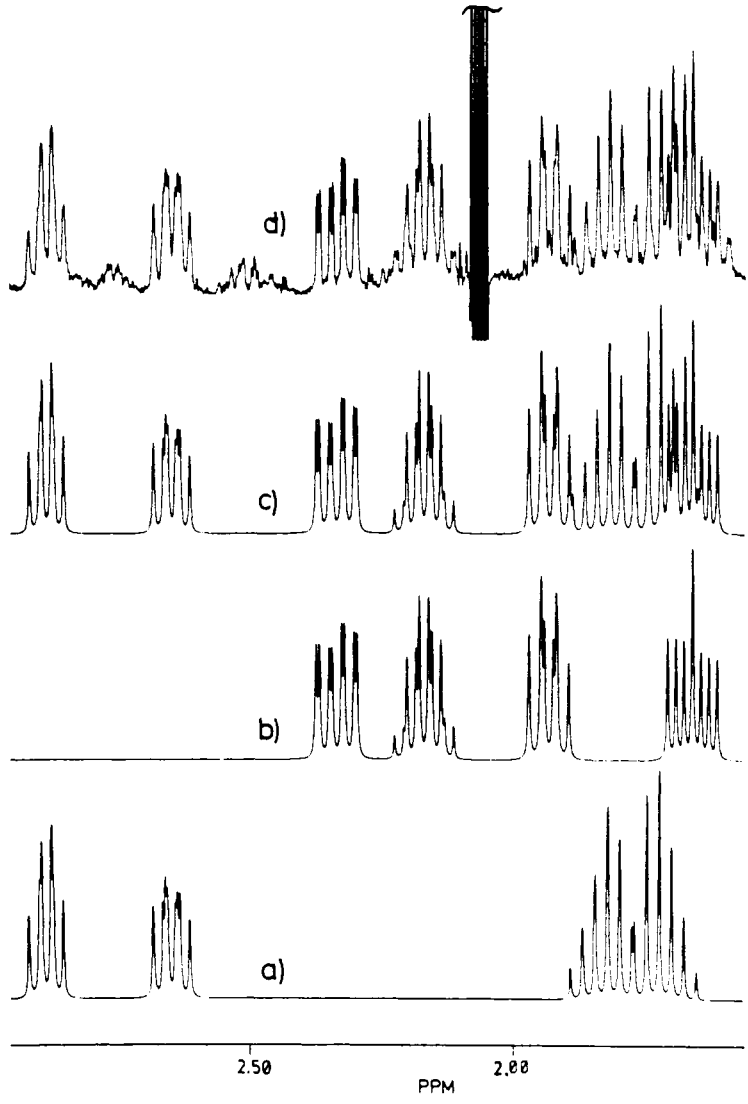


FIGURE 4. The low-frequency part of the spectral simulation of laciniatoside V [**1**], a, simulation, secologanin unit; b, simulation, loganin unit; c, superposition of a and b; d, measured spectrum.

The vicinal proton spin-spin coupling constants are sensitive measures of the conformation of the molecule, because of their dependence upon the dihedral angle (8). The two half-chair forms are the stable conformations of the pyran ring of the secologanin unit, because of the double bond. Consideration of $J_{1,9}$ allows determination of the conformation of this ring. As Figure 5 shows, the dihedral angle between the two protons is about 60° in conformer I and 180° in conformer II. Calculation of the predicted coupling constants for these two dihedral angles, according to a generalized Karplus equation that allows for the inductive effects of different substituents (8), gives values of 2.5 and 9.7 Hz, respectively. The measured value is 6.9 Hz, indicating that the pyran ring is in equilibrium between both conformers, with a slight excess of II over I. (An alternative explanation would be a flattening of the ring; however, flattening to the degree required appears improbable.)

TABLE 1. ^{1}H nmr Characteristics of Laciniatoside V [**1**].

Assignment[a]	δ[b]	m[c]	I[d]	Coupling constants (Hz)[e]
A-1-H	5.56	d	1	$^3J_{1,9}=6.9$
A-3-H	7.52	d	1	$^4J_{3,5}=1.0$
A-5-H	2.88	dt′	1	$^3J_{5,9}=5.5$; $^3J_{5,6a}=6.5$
A-6-H_a	1.83	ddt	1	$^3J_{6a,7}=7.0$; $^2J_{6a,6b}=-14.0$
A-6-H_b	1.72	dq′	1	$^3J_{5,6b}=7.0$; $^3J_{6b,7}=7.0$
A-7-H_2	3.58	o	2	
A-8-H	5.79	ddd	1	$^3J_{8,9}=8.1$; $^3J_{8,10E}=10.5$; $^3J_{8,10Z}=17.5$
A-9-H	2.64	dddt′	1	$^4J_{9,10E}=0.7$; $^4J_{9,10Z}=0.9$
A-10-H_E	5.21	ddd	1	$^2J_{10E,10Z}=-1.8$
A-10-H_Z	5.30	ddd	1	
B-1-H	5.00	t′	1	$^3J_{1,9}=6.5$; $^3J_{1,OH}=6.4$
B-3-H	7.42	d	1	$^4J_{3,5}=1.4$
B-5-H	3.13	dddd	1	$^3J_{5,6\alpha}=9.2$; $^3J_{5,6\beta}=7.1$; $^3J_{5,9}=8.9$
B-6-H_α	1.66	ddd	1	$^3J_{6\alpha,7}=4.8$; $^2J_{6\alpha,6\beta}=-14.0$
B-6-H_β	2.33	ddd	1	$^3J_{6\beta,7}=1.6$
B-7-H	5.24	td	1	$^3J_{7,8}=5.4$
B-8-H	2.17	pd	1	$^3J_{8,9}=7.1$; $^3J_{8,10}=7.1$
B-9-H	1.93	ddd	1	
B-10-H_3	1.09	d	3	
B-COOMe	3.67	s	3	
B-1-OH	6.40	d	1	
C-1-H	4.74	d	1	$^3J_{1,2}=8.0$
C-2-H	3.24	dd	1	$^3J_{2,3}=8.9$
C-6-H_a	3.88	dd	1	$^2J_{6a,6b}=12.0$
C-6-H_b	3.67	o	1	
C-3,4,5-H	3.3–3.5	m	3	
C-2,3,4,6-OH	4.0–4.4	m	4	

[a]See Figure 2 for numbering schemes in rings A and B. C denotes the glucose moiety. α, β, *E* and *Z* denote the stereochemical position of the proton in question; a and b denote a pair of CH_2 protons where stereochemical assignment cannot be made.

[b]Chemical shift in ppm versus internal TMS; in acetone-d_6.

[c]Multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet, o = overlapping with other signals. A prime indicates pseudo-behavior.

[d]Integral (number of protons).

[e]For units A and B, based on spectral simulation (see text). Partial analysis of C based on first-order inspection only. All values ±0.2 Hz (SD).

As Table 1 shows, at least one measured vicinal proton coupling constant is available for all bonds of the cyclopentane ring of the loganin unit. The values observed, particularly the high value of $J_{5,6\alpha}$ and the low value of $J_{6\beta,7}$, suggest that this ring exists to a large extent in a single dominant conformation. Assuming no deviations from ideal tetrahedral geometry for the ring carbons, the internal torsion angles of the ring can be

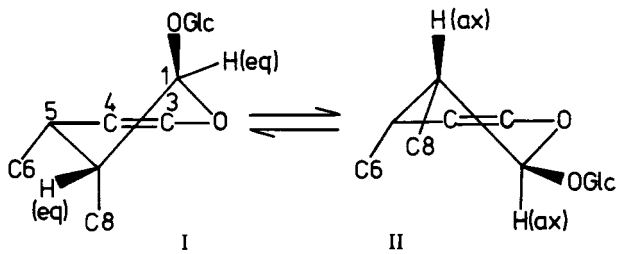


FIGURE 5. The probable conformational equilibrium of the pyran ring.

calculated from the measured coupling constants, using the generalized Karplus equation described above. The analysis is similar to that performed for lamalbide (9). There are always two torsion angles that fit the Karplus curve for a single coupling constant; however, because in the present case two coupling constants each are measured for the C-5–C-6 and C-6–C-7 bonds, this ambiguity can be removed. Results are summarized in Figure 6. Two values are given for torsion angles around C-5–C-6 and C-6–C-7, according to the two measured coupling constants; the variation within each pair is slight. The sum of the internal torsion angles is $-10°$. Given the errors inherent in this approach (estimated at $\pm 5°$ per bond) this is not significantly different from zero.

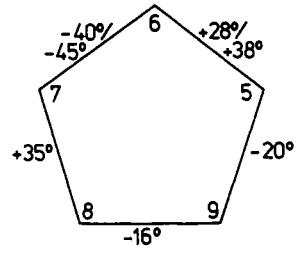


FIGURE 6. The internal torsion angles of the loganin cyclopentane ring. The angles are defined for the direction along the C-C bonds resulting in anticlockwise rotation around the ring as drawn.

The conformation thus characterized is the E_6 envelope (C-6 below the plane of the other four ring carbons), slightly distorted towards the $^7H_6$ half-chair (C-7 above, C-6 below the plane of the other three ring carbons). This in turn distorts the half-chair conformation of the condensed pyran ring towards the envelope conformation, but this change does not appreciably affect the 1-H–9-H dihedral angle. The measured value of $J_{1,9}$, 6.5 Hz, can again be interpreted in terms of a conformational equilibrium, in this case between the E_1 and the E^1 forms of the pyran ring. The condensed cyclopentane ring can adopt the conformation determined above for both conformations of the pyran ring.

The complete assignment of the ^{1}H-nmr spectrum was transferred into the ^{13}C-nmr spectrum by a 2D heteronuclear shift correlation measurement. Quaternary carbon assignments were based upon comparison with model compounds. Chemical shifts are given in Table 2. Taking into account the minor structural differences between the various bis-iridoids, the data are in close agreement with the previously reported ^{13}C chemical shifts of sylvestrosides I–III and cantleyoside, supporting the correctness of earlier, more empirical spectral assignments (4).

EXPERIMENTAL

ISOLATION.—Flowering *D. laciniatus* was collected at Szentendre Island, Hungary; it was identified at the Botanical Institute of Eötvös Loránd University of Sciences, Budapest. Voucher specimens (number 582) are deposited in the Herbarium of the Research Institute of Medical Plants, Budakalász, Hungary (Pilis Gene Reservation collection). The aerial parts of the plants (1200 g) were chopped into small pieces and extracted with EtOH. This extract was concentrated to an aqueous suspension, which was filtered, washed with $CHCl_3$ (200 ml), and chromatographed on Al_2O_3 (250 g, Woelm neutral) in H_2O. After

TABLE 2. ^{13}C-nmr Chemical Shifts of Laciniatoside V [**1**].[a]

Unit[b]	C-1	C-2	C-3	C-4	C-5	C-6
A	97.5		152.9	111.7	30.6	33.9
B	96.6		153.0	111.5	33.3	40.4
C	100.0	74.4	77.9[c]	71.4	77.7[c]	62.7
Unit	C-7	C-8	C-9	C-10	COO	Me
A	60.6	135.9	44.9	118.9	167.6	
B	77.3	41.3	48.1	14.3	168.3	51.3

[a]See Figure 2 for numbering schemes in rings A and B. C denotes the glucose moiety.

[b]Chemical shift in ppm versus internal TMS; in acetone-d_6.

[c]Assignments may be interchanged.

evaporation of the H_2O, the 35 g of material obtained was separated into fractions by countercurrent distribution using a CH_2Cl_2-MeOH-H_2O (14:15:7) two-phase solvent system (number of units 165, phase volume 100 ml, aqueous mobile phase, step number 354). Eluted fractions 22 and 23 were combined and evaporated, yielding 2.7 g of amorphous iridoid-containing fraction, which was further chromatographed on Si gel (100 g) with a CH_2Cl_2-MeOH-H_2O (32:5:0.5) mobile phase. Fractions (22 ml each) were collected; fractions 38–46 contained laciniatoside V [**1**]. This was finally purified on Si gel (32 g) with EtOAc-iPrOH-H_2O (20:2.5:1.25). Fractions (20 ml each) were collected; pure, amorphous laciniatoside V (30 mg) was obtained by combining fractions 37–42. Specific rotation was measured using a Carl Zeiss Polamat A instrument using Hg radiation at 546 nm. $[\alpha]^{27}_{546} = -98°$ (MeOH, $c = 0.67\%$); uv 237 nm (log ϵ 4.20); ir (KBr) 3600–3100 cm^{-1} (O-H), 1700 cm^{-1} (C=O, conjugated ester), 1640 cm^{-1} (C=C-O); found C 55.30%, H 6.47; calcd for $C_{27}H_{38}O_{14}$, C 55.29, H 6.53.

NMR SPECTRA.—These were recorded on a Bruker AM 300 spectrometer at frequencies of 300 MHz (^{1}H) and 75 MHz (^{13}C). Samples were prepared in acetone-d_6 at concentrations of 0.02 M (^{1}H) and 0.1 M (^{13}C). Internal TMS was used for a chemical shift reference. ^{13}C spin-echo gated-decoupled spectra were measured with a 7.2 msec evolution delay time, to determine the number of protons attached to the carbon atom corresponding to a given signal in the ^{13}C-nmr spectrum. [At this delay value, ^{13}C signals due to methyl and methine groups appear with normal phase and those due to methylene groups and quaternary carbons are inverted (10).] The homonuclear shift correlation (COSY) spectrum was measured with the standard Bruker DISNMR microprogram. A 90° mixing pulse, sine-squared apodization in both dimensions, and matrix symmetrization were employed. The heteronuclear shift correlation spectrum was also measured using standard spectrometer software, employing polarization transfer from ^{1}H to ^{13}C, with polarization transfer and rephasing delays of 3.8 ms and 2.2 ms respectively. Negative exponential apodization was used in the ^{13}C domain and sine-squared apodization in the ^{1}H domain.

Spectral simulations were performed by a Bruker spin simulation program (PANIC) separately for the iridoid and secoiridoid units of the molecule. The simulated spectra were then transferred into the main operating routine (DISNMR) and added together using the dual-display routine.

ACKNOWLEDGMENTS

We thank Dr. B. Dános, Eötvös Loránd University of Sciences, Budapest, for the plant identification. Financial support from the Natural Sciences and Engineering Research Council of Canada is also gratefully acknowledged.

LITERATURE CITED

1. L.J. El-Naggar and J.L. Beal, *J. Nat. Prod.*, **43**, 649 (1980).
2. T. Sevenet, C. Thal, and P. Potier, *Tetrahedron*, **27**, 663 (1971).
3. T. Endo, H. Susuki, and H. Taguchi, *Yakugaku Zasshi*, **96**, 246 (1976).
4. S.R. Jensen, S.E. Lyse-Petersen, and B.J. Nielsen, *Phytochemistry*, **18**, 273 (1979).
5. A. Kocsis, Z. Pál, B. Podányi, L. Szabó, and B. Tétényi, *IUPAC Int. Symp. Chem. Nat. Prod., 14th*, Poznan (1984).
6. A. Kocsis, B. Podányi, L. Szabó, and P. Tétényi, *Int. Conf. Chem. Biotech. Biol. Act. Nat. Prod., 4th*, Budapest (1987).
7. A.R. Battersby, E.S. Hall, and R. Southgate, *J. Chem. Soc. C*, 722 (1969).

8. C.A.G. Hasnoot, F.A.A.M. de Leeuw, and C. Altona, *Tetrahedron*, **36**, 2783 (1980).
9. P. Eigvet, S.R. Jensen, and B.J. Nielsen, *Acta. Chem. Scand. Ser. B*, **28**, 85 (1974).
10. A.E. Derome, "Modern NMR Techniques for Chemistry Research", Pergamon, 1987, p. 259.

Received 12 August 1988

RESEARCH CONGRESS ON NATURAL PRODUCTS

THIRTIETH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PHARMACOGNOSY
San Juan, Puerto Rico — August 6–10, 1989

Symposium on Natural Products and the Disease Condition

Documentation of Plant Use

Dr. Eloy Rodriguez, Professor, University of California at Irvine
"Selection and Chemistry of Medicinal Plants Used by Non-Human Primates"

Dr. Plutarco Naranjo, Secretary of Health and Professor, Central University, Quito, Ecuador
"Herbolaria of Precolumbian Times: Present and Future Perspectives" (Tentative)

Potential of Plant Use for Cancer and Acquired Immune Deficiency Syndrome (AIDS)

Dr. Yu Rencun, Chief Physician and Director of the Oncology Department, Bejing Hospital of Traditional Herbal Remedies, People's Republic of China
"Clinical Application of Chinese Herbal Remedies as Immunomodulators in the Treatment of Cancer"

Dr. John Cassady, Dean and Professor, The Ohio State University
"Natural Products as a Source of Potential Cancer Chemotherapeutic and Chemoprevention Agents"

Dr. David G.I. Kingston, Professor, Virginia Polytechnic Institute and State University, Blacksburg, Virginia
"Taxol—A Clinically Useful Anticancer Drug; Structure and Mechanistic Studies"

Dr. Kenneth Snader, Coordinator of Natural Products AIDS Testing, National Cancer Institute
"Recent Advances in Natural Products AIDS Research"
ASP Research Award Recipient

Symposium on the Biotechnology of Natural Products

Dr. David Evans, DNA Plant Technology, Cinnaminson, New Jersey
"Somaclonal Variation—Its Promise for the Industry of Natural Products" (Tentative)

Dr. G.N. Towers, University of British Columbia, Vancouver, British Columbia, Canada
"Role of *Agrobacterium* in the Production of Secondary Metabolites in Plants and Plant Tissue Culture"

This year's meeting will also include a workshop on "Simple Bioassays" which will be helpful in natural product research. Also, contributed papers covering all aspects of medicinal natural products research are invited as podium talks or poster sessions. Abstracts will be due May 15, 1989.

For further information please contact: Dr. Pedro I. Chavez
Scientific Program Chairman
School of Pharmacy
University of Puerto Rico
G.P.O. Box 5067
San Juan, P.R. 00936
Phone: (809) 758-2525 Extension 5316 or 5410