Ergobalansine/Ergobalansinine, a Proline-Free Peptide-Type Alkaloid of the Fungal Genus Balansia, Is a Constituent of Ipomoea piurensis

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ERGOBALANSINE/ERGOBALANSININE, A PROLINE-FREE PEPTIDE-TYPE ALKALOID OF THE FUNGAL GENUS *BALANSIA*, IS A CONSTITUENT OF *IPOMOEA PIURENSIS*[1]

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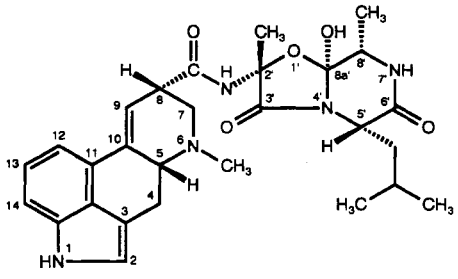
ABSTRACT.—A proline-free ergopeptine-type alkaloid, ergobalansinine [**1**], and three simple ergoline alkaloids, chanoclavine-I, ergine, and lysergic acid α-hydroxyethylamide, were isolated from the seeds of *Ipomoea piurensis*. The structure of **1** was established on the basis of spectral data. All compounds are also present in the other epigeal parts in contrast to the roots.

The Convolvulaceae are characterized by the occurrence of structurally diverse types of alkaloids: tropanes (2,3), indolizidines (4), pyrrolizidines (5), and unique serotonin-hydroxycinnamic acid conjugates (6,7) are documented in the literature. Furthermore, it is well-known that this family is the only source thus far of ergoline alkaloids in higher plants. Even ergopeptine-type compounds, characteristic metabolites of the ergot fungus *Claviceps purpurea* Tulasne, have been discovered in different species of the Convolvulaceae. Previously this has only been proved by isolation of ergosine, a congener of ergotamine, in two cases (8,9).

Our phytochemical investigation on the seeds of *Ipomoea piurensis* O'Donell, a morning glory species of tropical South America, collected near Guayaquil, Ecuador, resulted in the isolation of chanoclavine-I (10), ergine, and lysergic acid α-hydroxyethylamide (11) together with the unusual peptide-type alkaloid ergobalansinine [**1**]. This natural product and its 8*R*-isomer, probably the naturally occurring ergobalansine, both bearing a rather uncommon peptide moiety, have already been isolated from two *Balansia* species, fungi in the family Clavicipitaceae living as endophytes in sandbur grass (*Cenchrus echinatus*) and the sedge *Cyperus virens* (12).



1

The identification of **1** was achieved by eims, hrms, fabms, and ^{1}H-nmr spectroscopy. The eims of **1** gave a molecular ion peak $[M]^+$ at *m/z* 521 which was confirmed by positive- and negative-ion fabms. From the hrms, the molecular formula could be deduced as $C_{28}H_{35}N_5O_5$. Other significant fragments at *m/z* 337.1414 $[C_{19}H_{19}N_3O_3]$, *m/z* 319.1321 $[C_{19}H_{17}N_3O_2]$, *m/z* 267.1375 $[C_{16}H_{17}N_3O]$, and *m/z* 184.1216 $[C_9H_{16}N_2O_2]$ were also present. The ion at *m/z* 267 corresponds to free lysergic acid amide, and is frequently observed with this type of alkaloid. The ^{1}H-nmr spectrum (Table 1) showed characteristic signals for an indole moiety: δ 7.95 ppm (1H, br s, H-1), 7.16 ppm (1H, dd, J_1=7.5, J_2=7.0 Hz, H-13), 7.20 ppm (1H, d, J=7.5 Hz, H-12/H-14), 7.25 ppm (1H, d, J=7.0 Hz, H-12/H-14), and 6.93 ppm (1H, m, H-2). All other signals of the lysergic acid moiety could be assigned by decoupling experiments. Comparison of the chemical shift of H-8 with data of ergotamine and its C-8 epimer suggested the isolated alkaloid to be a derivative of isolysergic

[1]Part 3 in the series "Phytochemistry and Chemotaxonomy of the Convolvulaceae." For Part 2, see Henrici *et al.* (1).

TABLE 1. ^{1}H-Nmr Data of Ergobalansinine [**1**] ($CDCl_3$, 400 MHz, δ values, *J* in Hz).

Proton	1		
H-1	7.95 br s	1H	
H-2	6.93 m	1H	
H-4a	3.61 dd	1H	(14.5, 5.5)
H-4b	2.65 ddd	1H	(14.5, 12.0, 2.0)
H-5	3.25 m	1H	
H-7a	3.15 br d	1H	(12.0)
H-7b	2.77 dd	1H	(12.0, 3.5)
H-8	3.07 m	1H	
H-9	6.51 br d	1H	(6.5)
H-12	7.20 br d	1H	(7.5)*
H-13	7.16 dd	1H	(7.5, 7.0)
H-14	7.25 br d	1H	(7.0)*
N-6-CH_3	2.63 s	3H	
H-5′	4.46 dd	1H	(7.0, 6.5)
H-8′	3.55 dq	1H	(6.5, 2.0)
CH_2-5′	1.90 m	2H	
CH_2-*CH*-5′	1.95 m	1H	
$CH_2CH(CH_3)_2$-5′	0.95 d	3H	(6.5)
	0.96 d	3H	(6.5)
CH_3-2′	1.50 s	3H	
CH_3-8′	1.33 d	3H	(6.5)
OH-8a′	5.48 s	1H	
NH-7′	6.79 d	1H	(2.0)
CO-NH	9.85 s	1H	

*Interchangeable.

acid (12,13). The ^{1}H-nmr spectrum of the unusual peptide moiety showed a C-2′-methyl singlet at δ 1.50 ppm as in ergotamine. Furthermore, we could observe H-5′ at δ 4.46 ppm (1H, dd, J_1=7.0 Hz, J_2=6.5 Hz) coupled to the methylene protons at δ 1.90 ppm (2H, m) of an isobutyl group. There were no signals that could be assigned to the methylene protons of a proline residue, which usually is a constituent of ergopeptine alkaloids. Instead, the spectrum showed a methyl doublet at δ 1.33 ppm (3H, d, *J*=6.5 Hz) coupled to H-8′. Thus, the proline moiety must be replaced by a second alanine residue. Indeed, all data were in agreement with those of ergobalansinine (12). Concerning the stereochemistries of the positions 2′, 5′, 8′, and 8a′, comparison of the chemical shift values with published data (12,13) indicated that they should be the same as for the regular series of ergopeptines. Nevertheless, the configurations at these centers remain to be determined.

The 8-epimer of **1**, the 5*R*,8*R*-lysergic acid derivative ergobalansine, could not be isolated because of separation problems with other constituents. However, it is well-known that 5*R*,8*R*-lysergic acid derivatives are the naturally occurring compounds which spontaneously epimerize in part to the corresponding 5*R*,8*S*-isomers in the presence of H_2O. Therefore, they are always also present in extracts as in the case of the sclerotia of *Claviceps purpurea* (14,15).

Certain species of the Convolvulaceae are capable of synthesizing additional compounds related to the regular proline-containing ergopeptines such as ergosine, which is previously known from *Claviceps purpurea*. Now the very unusual ergobalansine/ergobalansinine, the first proline-free alkaloid of this type in nature, has been established as a constituent of this plant family. Furthermore, this is, besides cycloclavine (16), only the second ergoline compound known to occur in the Convolvulaceae but that has not yet been discovered in a *Claviceps* species. The isolation of **1** from the seeds of *I. piurensis* is the third real proof for the occurrence of ergopeptines in higher plants and could be of chemotaxonomic value. In all those cases in which "ergosine" or "ergotamine" have been characterized only by tlc comparison with an authentic sample (17–20), reinvestigations seem to be necessary because these ergopeptines and ergobalansine/ergobalansinine show very similar chromatographic behavior on the usual absorbents.

The simple ergolines, chanoclavine-I, ergine, and lysergic acid α-hydroxyethylamide, also isolated from the seeds of *I. piurensis*, are typical constituents of several species of the Convolvulaceae belonging mostly to species in the genera *Ipomoea* and *Argyreia* (21,22). These simple ergolines as well as **1** and its 8-epimer are also present in the leaves and stems of *I. piurensis*, but not in the roots.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-Nmr spectra were obtained in $CDCl_3$ on a Bruker AM-400 nmr spectrometer. Eims, hrms, and fabms

were recorded using MAT-711 and CH_5-DF Finnigan spectrometers.

PLANT MATERIAL.—The seeds of *Ipomoea piurensis* were collected near Guayaquil, Ecuador in December 1991. The epigeal parts and roots were harvested from the greenhouse of the Institut für Pharmazeutische Biologie, Freie Universität Berlin, in 1993, where a voucher specimen of the plant is deposited.

EXTRACTION AND ISOLATION.—Ground seeds of *I. piurensis* (500 g) were defatted with petroleum ether (4 liters) in a Soxhlet apparatus for 6 h. The dried powder was stirred with MeOH (2.5 liters). After evaporation the residue was partitioned between H_2O and $CHCl_3$. Finally, the aqueous layer was alkalinized (3% aqueous NH_3) and again extracted with $CHCl_3$ to afford the crude alkaloid fraction (127 mg). Separation of the alkaloids was achieved by centrifugal tlc on Si gel [$CHCl_3$-MeOH-cyclohexane (100:2:10)] to give **1** (1 mg) in addition to chanoclavine-I (12 mg), ergine (2.5 mg), and lysergic acid α-hydroxyethylamide (2.5 mg). Spectroscopic data and chromatographic behavior of the simple ergolines were identical with literature (10,11) and authentic samples.

Ergobalansinine [**1**].—Colorless oil, eims (80 eV) *m/z* 521 (M^+, 0.5), 337 (2), 319 (4), 267 (73), 221 (25), 184 (42), 141 (20), 128 (100), 113 (28), 99 (20), 70 (18), 43 (56); hreims (80 eV) *m/z* 337.1414 [calcd for $C_{19}H_{19}N_3O_3$, 337.1426], 319.1324 [calcd for $C_{19}H_{17}N_3O_2$, 319.1321], 267.1375 [calcd for $C_{16}H_{17}N_3O$, 267.1372], 184.1216 [calcd for $C_9H_{16}N_2O_2$, 184.1212]; negative-ion fabms *m/z* $[M-H]^-$ 520; positive-ion fabms *m/z* $[M+H]^+$ 522; ^1H nmr, see Table 1.

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