CATHARANTHUS ALKALOIDS XXXVII. 16-EPI-*Z*-ISOSITSIRIKINE, A MONOMERIC INDOLE ALKALOID WITH ANTINEOPLASTIC ACTIVITY FROM *CATHARANTHUS ROSEUS* AND *RHAZYA STRICTA*[1]

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ABSTRACT.—16-Epi-*Z*-isositsirikine (**1**) has been isolated from the leaves of *Catharanthus roseus* and *Rhazya stricta* and identified through a combination of spectral interpretation and chemical correlation. The compound displayed antineoplastic activity in the KB test system *in vitro* and the P-388 test system *in vivo*.

As part of our continuing studies of the antineoplastic principles of the apocynaceous plants *Catharanthus roseus* (L.) G. Don (1-4) and *Rhazya stricta* Decsne (5), we have isolated, through bioactivity-directed fractionation,[2] a monomeric indole alkaloid **1**, possessing activity in both the KB *in vitro* and P-388 lymphocytic leukemia *in vivo* test systems. The structure of the isolate, which possesses the rare *Z*-configuration for the 19,20-double bond, was determined through spectral interpretation and comparison with the reduction products of geissoschizine (**2**).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were obtained by means of a Kofler hot-stage apparatus and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrometer. The ir spectra were determined on a Perkin-Elmer, model 710 spectrometer. Proton nmr spectra were recorded in $CDCl_3$ or acetone-d_6 on a Varian model T-60A spectrometer with a Nicolet TT-7 Fourier Transform attachment operating at 60 MHz or at 270 MHz on a Brücker WH-270 instrument. Tetramethylsilane was used as an internal standard, and chemical shifts are recorded in δ-units (ppm). Mass spectra were obtained with an AEI MS 902 or a Varian MAT112S double focusing spectrometer operating at 70 eV, and optical rotations were measured with a Perkin-Elmer 241 spectrometer.

PREPARATION OF ALKALOID FRACTIONS FROM *R. STRICTA*.—The source, identification, and processing of the leaves of *Rhazya stricta* Decsne used in this study have been described previously (5).

SEPARATION OF THE GRADIENT pH 6.7 ALKALOID FRACTION.—Fraction 2 was subjected to gradient pH separation according to the method of Svoboda (2), with only minor modification. Seven fractions, 2A-2G, were obtained (5).

Fraction 2-F (3.4 g, KB, ED_{50} 0.97 μg/ml) was chromatographed over a column containing silica gel[3] (100 g) packed in chloroform. Fraction 4 from the column was taken to dryness *in vacuo* and triturated with a little methanol to afford a solid that crystallized as pale yellow needles (**1**, 3.7 mg, 0.000016%) from methanol.

PREPARATION OF ALKALOID FRACTIONS FROM *C. ROSEUS*.—The source, identification, and processing of the leaves of *Catharanthus roseus* (L.) G.Don used in this study have been described previously (7).

[1]For part XXXVI in this series see reference (1).

[2]Fractions and compounds were evaluated for anticancer activity according to established protocols (6).

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SEPARATION OF THE GRADIENT pH 5.5 ALKALOID FRACTION FROM F-010.—Preliminary tlc experiments, using the solvent systems ethyl acetate-absolute ethanol (9:1), chloroform-methanol (95:5) and benzene-triethylamine (9:1), revealed at least ten constituents in the alkaloid fraction pH 5.5. Chromatography of a sample of the crude alkaloid fraction (15.8 g) on a column of silica gel PF_{254}[3] (600 g) packed in chloroform-benzene (1:1) was initiated by elution with the same solvent system, followed by solvents of increasing polarity through the addition of chloroform and methanol. Grouping of the eluted fractions was accomplished on the basis of similar tlc patterns as well as chromogenic reactions of resolved alkaloids to the ceric ammonium sulfate (CAS) detecting reagent. Fractions were monitored throughout by concomitant bioassay. A total of 113 fractions were collected, analyzed, and combined into 14 groups.

Preliminary tlc of column fractions 68-76 and 77-82 revealed that they contained a major constituent giving a yellowish-green color reaction with the CAS spray reagent. Each of the combined fractions was separately taken to dryness *in vacuo*, dissolved in chloroform, and stored at 0°. After several days, pale yellow needles of **1** were deposited and recrystallized to afford from combined fractions 68-72 and 77-82 a total of 84 mg (0.00002%).

PHYSICAL PROPERTIES OF THE ISOLATE.—The isolate displayed the following physical and spectral properties, mp 181°, $[\alpha]^{25}D-41°$ (c 0.1, MeOH); ir, ν max (KBr) 3320, 2920, 2850, 1710, 1430, 1370, 1280, 1160, 1050, 980 and 730 cm^{-1}; uv, λ max (MeOH) (log ϵ) 225 (4.10), 280 (3.90) and 290 nm (3.75); ^{1}H-nmr, δ (270 MHz, acetone-d_6) 1.49 (dd, 1H, J=12, 12 Hz, 14β-H), 1.69 (d, 3H, J=6 Hz, 18-H), 2.25 (dt, 1H, J=4, 12 Hz, 14α-H), 2.62 (m, 1H, 5α-H), 2.75 (d, 1H, J=12 Hz, 6α-H), 2.89 (br s, 3H, 15, 16, 21α-H), 3.02 (m, 1H, 6β-H), 3.13 (m, 1H, 5β-H), 3.46 (br d, 1H, J=12 Hz, 3-H), 3.64 (s, 3H, -CO_2CH_3), 3.87 (m, 2H, 17-H_2), 3.88 (br d, 1H, J=12 Hz, 21β-H), 5.46 (q, 1H, J=6 Hz, 19-H), 6.99 (m, 2H, 10, 11-H), 7.27 (d, 1H, J=7 Hz, 12-H), 7.39 (d, 1H, J=7 Hz, 9-H), and 8.02 (s, 1H, N-H, exchanged with D_2O); ms, *m/z* (%) 354 (M^+, 99), 353 (75), 339 (6), 336 (1), 325 (6), 324 (8), 323 (24), 295 (6), 252 (34), 251 (100), 250 (11), 249 (22), 237 (10), 223 (13), 184 (8), 171 (21), 170 (23), 169 (43), 168 (12), 167 (6), 156 (24), 154 (11), 144 (14), 143 (11), 142 (7), 130 (8), 129 (9), 115 (10), 93 (5), 91 (6), 83 (7), 81 (5), 79 (8), and 77 (8). Mass measurement observed 354.1952; calcd for $C_{21}H_{26}N_2O_3$ 354.1943.

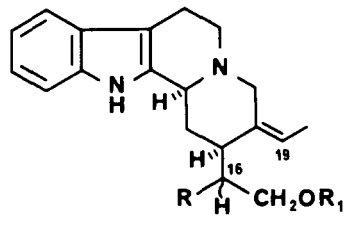
ACETYLATION OF **1**.—A solution of the isolate (**1**, 10 mg) in pyridine-acetic anhydride (3 ml, 1:2) was kept at room temperature overnight. The reaction mixture, when poured into cold water, extracted with chloroform (3 x 10 ml), dried over Na_2SO_4 and evaporated *in vacuo* afforded a residue of **3** (5.5 mg); ^{1}H-nmr, δ (60 MHz, $CDCl_3$) 1.47 (d, 1H, J=6,7 Hz, 18-H), 2.00 (s, 3H, 17-$OCOCH_3$), 3.70 (s, 3H, -CO_2CH_3), 4.29 (m, 2H, 17-H_2), 5.68 (m, 1H, 19-H), and 7.54 (m, 5H, 9, 10, 11, 12 and NH).

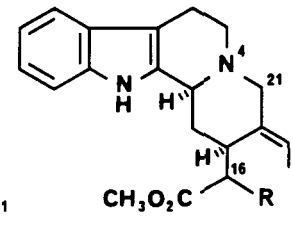
LiA1H_4 REDUCTION OF **1**.—A solution of the isolate (**1**, 3 mg) in anhydrous ether (5 ml) was treated with a small quantity (approximately 1 mg) of LiA1H_4 and the mixture stirred under N_2 at room temperature for 2 h. The reaction was terminated by the slow addition of 2 ml of water, followed by extraction with chloroform (3 x 3 ml), and was dried over Na_2SO_4 and evaporated. The product (2 mg) was the diol **4**, displaying ms, *m/z* (%) 326 (M^+, 83), 309 (17), 295 (26), 261 (13), 251 (84), 247 (38), 246 (41), 244 (16), 183 (36), 170 (57), 169 (100), and 156 (51).

KETALIZATION OF DIOL **4**.—To a solution of the diol **4** (2 mg) in dry acetone (2 ml) was added *p*-toluene sulfonic acid (trace), and the reaction allowed to stand at room temperature overnight. The reaction mixture was poured into cold water, basified with aqueous ammonia, extracted with chloroform (3 x 3 ml), and dried over Na_2SO_4. On removal of chloroform *in vacuo*, quantitative recovery of the acetonide derivative **5** was achieved; ms, *m/z* (%) 366 (18), 350 (18), 308 (8), 307 (1), 272 (13), 261 (4), 252 (26), 251 (53), 250 (32), 249 (100), 247 (16), 237 (8), 235 (10), 170 (8), 169 (13), 156 (5), and 115 (50).

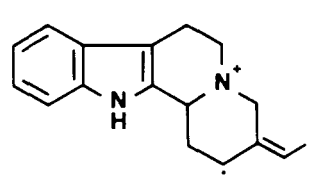
$NaBH_4$ REDUCTION OF GEISSOSCHIZINE (**2**).—To a solution of geissoschizine (**2**, 2 mg) in methanol (5 ml), $NaBh_4$ was added and the mixture kept at room temperature overnight. After the usual work-up, the reaction mixture displayed two spots under the solvent system chloroform-methanol (95:5) on silica gel, eluting twice. These compounds were identified as *E*-isositsirikine (Rf 0.39) (**6**) and 16-epi-*E*-isositsirikine (Rf 0.29) (**7**). No correspondence was observed with the isolate, which displayed Rf 0.35. Similar separation of the isomers could be achieved by tlc on silica gel eluting with benzene-triethylamine (86:14).

STRUCTURE ELUCIDATION OF 16-EPI-*Z*-ISOSITSIRIKINE (**1**).—The characteristic uv spectrum of the isolate was consistent with a 2,3-disubstituted indole chromophore (8) and the ir spectrum indicated the presence of NH, OH, and saturated ester functionalities. The ^{1}H-nmr spectrum at 270 MHz was obtained in acetone-d_6, because the compound was poorly soluble in $CDCl_3$. It indicated the presence of a vinyl methyl group as a doublet (J=6 Hz) at 1.69 ppm, coupled with a one-proton quartet at 5.46 ppm; these chemical shifts and multiplicities are consistent with an ethylidene side chain. A two-proton complex pattern at 3.88 ppm was attributed to a primary hydroxy group, shifting to 4.29 ppm in the acetate derivative. A methoxy-carbonyl group was evident from a three-proton singlet at 3.64 ppm. Signals in the aromatic region confirmed the 2,3-disubstituted nature of the indole nucleus.

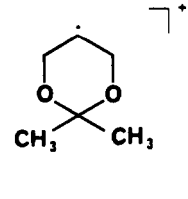




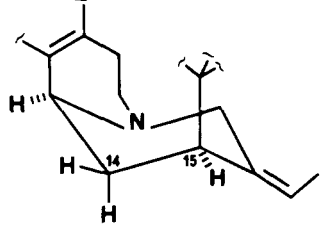
		R	R_1		R
1	16S	CO_2CH_3	H	2	H, CHO
3	16S	CO_2CH_3	Ac	6	16R-H, CH_2OH
4	16S	CH_2OH	H	7	16S-H, CH_2OH
5	16S	$-CH_2-O-C(CH_3)_2-$		9	H, CH_2OH no stereochemistry
11	16R	CO_2CH_3	H	12	H, CHO $\Delta^{4,21}$



8 m/z 251



10 m/z 115



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Peak matching of the molecular ion at *m/z* 354 indicated a molecular formula of $C_{21}H_{26}N_2O_3$, and analysis of the base peak at *m/z* 251 (**8**) indicated that it contained no oxygen. Biogenetic reasoning and the presence of ms fragmentation ions at *m/z* 184, 170, 169, and 156 suggested a β-carboline nucleus and a gross structure **9** for this alkaloid.

Further evidence for the nature and location of the functional groups was achieved by $LiAlH_4$ reduction at room temperature for two hours to afford a diol **4** exhibiting a molecular ion at *m/z* 326. The 1,3-dihydroxy relationship in diol **4** was confirmed by the formation of an acetonide derivative **5**, which, in the ms showed a characteristic loss of 115 amu (interpreted as **10**) to afford the base peak at *m/z* 251. The isolate was, therefore, deduced to be of the Corynanthe type in which it remained to determine the stereochemistry at C-3, C-16, and C-19. The assumption is that C-15 has the same absolute stereochemistry in all indole alkaloids (9).

The stereochemistry at C-3 was readily deduced as α, because the cd spectrum, in methanol, displayed a positive Cotton effect in the region 250-300 nm. This is in agreement with indole alkaloids in this series having the C-3 hydrogen in an α-configuration (10,11).

There are two known, natural indole alkaloids with these structure limitations, isositsirikine (**6**) (8) and 16-epi-isositsirikine (**7**) (12). Recently, Husson *et al.* (13) reported on the high-field ^{1}H-nmr spectral parameters for these compounds and their 19-isomers **1** and **11**, in which the 19, 20-double bond now has the *Z*-configuration. All four isomers were produced from the $NaBH_4$ reduction in methanol of the equilibrated mixture obtained from 4,21-dehydrogeissoschizine hydrochloride (**12**) (14). A distinction between *E*-isositsirikine (**6**) and 16-epi-*E*-isositsirikine (**7**) was made previously by chemical correlation (15). Husson *et al.* observed that the 3-H in the *E*-isositsirikines (**6** and **7**) appeared downfield of the correspond-

ing signal in the *Z*-isositsirikines (**1** and **11**) (13). Because of a strong interaction in the "normal" conformation (*trans*-quinolizidine) between C-18 of the *E*-ethylidene chain and the group at C-15, the predominant conformation of the *E*-isositsirikines would be **13**. This interaction is absent in the *Z*-isositsirikines (**1** and **11**).

In the isolate, the observation of a one-proton doublet with fine splitting at 3.46 ppm, (*i.e.*, at higher field than 3.85 ppm) rising from the 3-H indicates the possibility of a 19,20-*Z*-configuration (13). This was substantiated when $NaBH_4$ reduction of an authentic sample of geissoschizine (**2**) (*E*-configuration)[4] afforded **6** and **7**, which were chromatographically different from the isolate on silica gel, using the solvent systems chloroform-methanol (95:5) and benzene-triethylamine (86:14).

Another major difference between *E*-isositsirikines (**6** and **7**) and *Z*-isositsirikines (**1** and **11**) is the chemical shift of 14β-H. Owing to the interaction between C-18 and 14β-H in conformation **13** of *E*-isositsirikines (**6** and **7**), the 14β-H resonates further downfield in the *E*-isomers that in the *Z*-isomers. A one-proton doublet of doublets at 1.46 ppm (J=12, 12 Hz) was consistent with the chemical shift of this proton in 16-epi-*Z*-isositsirikine (**1**). Indeed, the resonances for the protons H-3, H-5α, H-5β, H-14α, H-15, H-16, H-18, and H-21α, all corresponded closely with those observed (13) for **1** rather than the 16-epimer **11**. On this basis the structure of the isolate was tentatively assigned as 16-epi-*Z*-isositsirikine (**1**).

BIOLOGICAL ACTIVITY OF **1**.—The isolate (NSC-338932) was evaluated for antineoplastic activity according to established protocols (6). It was found to be cytotoxic in the KB test system *in vitro* (ED_{50} 1.20 μg/ml) and at doses in the range 2.5-10 mg/kg to display good, reproducible activity (T/C≥150%) in the P-38 lymphocytic leukemia test system *in vivo*.

DISCUSSION

Both *Catharanthus roseus* (16) and *Rhazya stricta* (17) have been widely studied phytochemically, and the former plant has yielded a number of *in vivo* active antineoplastic agents, of which the bisindole akaloids vincaleukoblastine (VLB) and leurocristine (VCR) are in clinical use (18).

We report here the structure elucidation of a new alkaloid, tentatively identified as 16-epi-*Z*-isositsirikine (**1**), which was isolated through bioactivity-directed fractionation of extracts from both *C. roseus* and *R. stricta*. The structure assignment was made on the basis of interpretation of spectral data and comparison with the $NaBH_4$reduction product of geissoschizine (**2**).

In the recent past we have isolated a number of new bisindole alkaloids from *C. roseus* displaying antineoplastic activity (2-4). However, this is the first report of a monomeric indole alkaloid displaying *in vivo* antineoplastic activity to be isolated from either *C. roseus* and *R. stricta*. It is also the first isolation of a simple 19,20-unsaturated indole alkaloid possessing the 19,20-*Z*-configuration, although evidence for the existence of such compounds is abundant (13,19,20).

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