ANTICANCER INDOLE ALKALOIDS OF *RHAZYA STRICTA*

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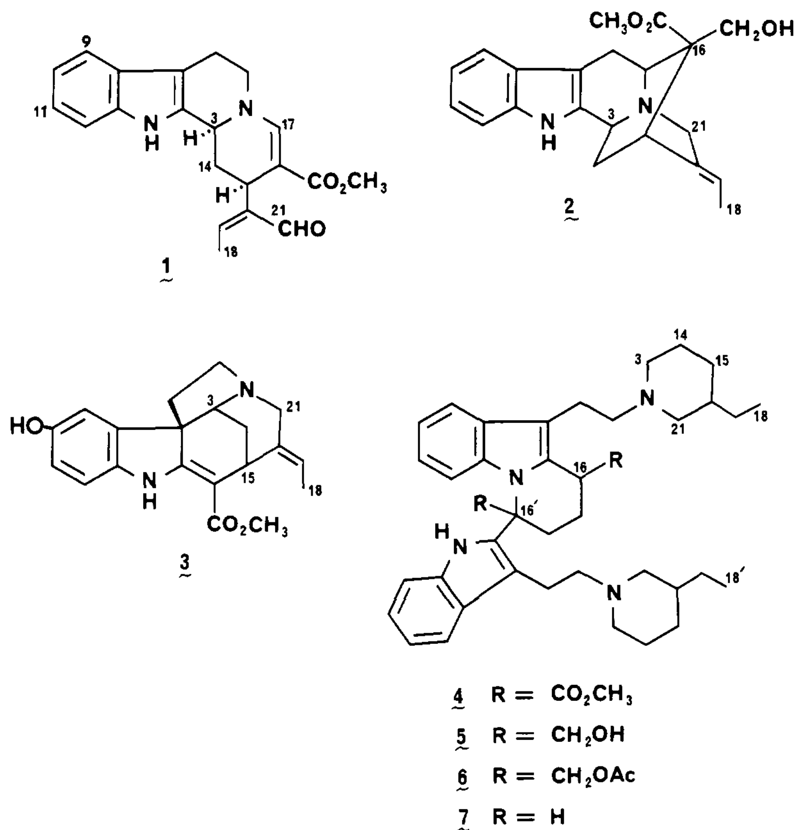
ABSTRACT.—The leaves and roots of *Rhazya stricta* (Apocynaceae) yielded four indole alkaloids. Three of these isolates, vallesiachotamine (**1**), sewarine (**3**) and tetrahydrosecamine (**4**), displayed cytotoxic activity; polyneuridine (**2**) was not reported previously from this species. Tetrahydrosecaminediol (**5**), its acetate **6**, and didemethoxycarbonyl-tetrahydrosecamine (**7**) were prepared from tetrahydrosecamine (**4**) by chemical reactions. The diol **5** was highly cytotoxic.

The genus *Rhazya*, in the indole alkaloid-rich family Apocynaceae, is comprised of only two species (1), *Rhazya stricta* Decsne., a small erect shrub growing in the north west of the Indian subcontinent (2,3), and *Rhazya orientalis* A.DC., native to northwestern Turkey and Western Thrace.

No medicinal uses of *R. orientalis* have been reported, but *R. stricta* is a reputed bitter tonic and curative for chronic rheumatism (4–7).

The presence of alkaloids in *R. stricta* was first detected by Hooper (8), and by 1945 its rich alkaloid content was established. Since then the total number of indole alkaloids isolated from *Rhazya* species has risen to more than fifty, and this work has been summarized by Chatterjee and co-workers (9).

Indian workers, in 1972, demonstrated that extracts of *R. stricta* showed a marked leucopenic effect in rats when given orally (20 mg/kg) and that a single i.p. injection (15 mg/kg) significantly reduced the white blood cell count for 7–10 days (10). We were intrigued by this activity, and this paper reports some



of our preliminary work on the isolation of a number of biologically interesting alkaloids from this plant.

EXPERIMENTAL[1]

PLANT MATERIAL.—The *Rhazya stricta* Decsne. (Apocynaceae) leaves and roots used in this study were obtained from India by the Economic Botany Laboratory, USDA, and a sample has been deposited in the herbarium of the National Arboretum, Beltsville, MD.

PREPARATION OF ALKALOID FRACTIONS FROM *R. stricta* LEAVES.—A sample of coarsely milled leaves (4.5 kg) was extracted continuously for 24 hr. in a percolator with petroleum ether (40–80°). The extract was concentrated and removed from the extractor, and a fresh charge of solvent was added. This procedure was repeated four times. Pooling of the four concentrates followed by evaporation *in vacuo* afforded 15.7 g of Fraction 1.

The extracted *R. stricta* leaves were air dried, macerated with methanol, and exhaustively extracted by repeated maceration and percolation. The combined percolates (ca. 20 liters), when concentrated, afforded 41.7 g of Fraction II.

When the residual methanol was removed from the leaves of the plant and extracted with chloroform, 13.1 g of Fraction III was obtained.

TABLE 1. Cytotoxicity of *Rhazya stricta* fractions.

Fractions	KB (ED_{50} μg/ml)[a]
I	>100
II	19.0
III	20.0
IV	52.0
V	17.0
VI	55.0
VII	10.0

[a]An active fraction is one that exhibits $ED_{50} \leqq 20$ μg/ml (12).

GRADIENT PH SEPARATION OF FRACTION II.—Fraction II was subjected to a gradient pH separation according to the method of Svoboda (11) with only minor modifications. Fraction II (41.7 g) was dissolved in a minimum volume of ethylene dichloride and extracted into 2 liters of 0.2 M tartaric acid solution by gentle warming on a steam bath and removal of ethylene dichloride *in vacuo*. Extraction of this acidic filtrate (pH 2.7) was effected with three separate volumes of benzene, and the aqueous layer was adjusted to pH 3.0 with ammonium hydroxide solution followed by three extractions with benzene. This procedure was continued to increasing pH with additions of ammonium hydroxide solution until a final pH 9.0 was attained. Benzene extracts at each pH level were combined and taken to dryness, and the groupings were made according to table 2.

TABLE 2. Gradient pH separation of Fraction II.

Group	pH	Wt (g)	KB results[a] (ED_{50} μg/ml)
II-A	3.0	3.1	18.0
II-B	3.5	2.7	26.0
II-C	4.0	3.7	14.0
II-D	5.0	2.2	15.0
II-E	5.5	3.6	11.0
II-F	6.7	3.4	0.97
II-G	9.0	2.3	57.0

[a]An active fraction is one that exhibits $ED_{50} \leqq 20.0$ μg/ml (12).

PREPARATION OF ALKALOID FRACTIONS FROM *R. stricta* ROOTS.—The dried, milled *R. stricta* roots (22.5 kg), when extracted with petroleum ether (40–80°), yielded Fraction IV (15.7 g).

[1]Melting points were determined by means of a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrometer. The ir spectra were determined on Perkin-Elmer, model 710 spectrometer. Proton nmr spectra were recorded in $CDCl_3$, CD_3COCD_3 and 2% DCl-D_2O on a Varian model T-60 A instrument with a Nicolet TT-7 Fourier Transform attachment and a 270 MHz Bruker instrument. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ-units (ppm). High resolution mass spectra were obtained with a Varian MAT 112S double focusing spectrometer operating at 70 ev.

After being air dried, the defatted roots were exhaustively extracted with methanol (150 liters). The extract was concentrated *in vacuo* to a syrupy consistency and was partitioned between distilled water (1 liter) and chloroform (5 liters). The chloroform soluble fraction was dried (Na_2SO_4) and concentrated *in vacuo* at 40°, and the residue was triturated with 2% tartaric acid (2 liters). The neutral fraction V, after processing, weighed 43.7 g. The acid solution was chilled and rendered alkaline (pH 8.5) with a saturated solution of sodium carbonate. The alkaline solution was then extracted with three 1 liter volumes of chloroform. The chloroform extracts, when pooled, washed with water, dried over Na_2SO_4, filtered and taken to dryness *in vacuo* yielded 60.2 g of crude tertiary bases of Fraction VI.

Fraction VII (20.5 g) was obtained from the aqueous alkaline solution after it was made neutral and the water was removed through lyophilization.

Cytotoxic evaluation of alkaloid fractions.—Fractions I–VII were evaluated for cytotoxicity against Eagle's KB carcinoma of the nasopharynx in cell culture according to established protocols (12). The results are presented in table 1. Further separation was made on the basis of the activity of the fractions.

Characterization of vallesiachotamine (**1**) and polyneuridine (**2**).—Fraction II-F (3.4 g) was chromatographed over a column containing silica gel[2] (100 g) packed in chloroform. A total of 15 fractions (50 ml each) was collected from the column and combined on the basis of their tlc patterns.

Fractions 7–9 from the column were combined and subjected to preparative tlc with methanol as the eluent. Two bands were removed. The band of Rf 0.76, when purified, afforded an amorphous gum (13.0 mg), mp 244°; ir, νmax (KBr) 3440 (br s, NH or OH), 2960 (w), 2840 (w), 1690 (s), 1610 (s), 1450 (m), 1430 (m), 1340 (m), 1300 (m), 1190 (m), 1105 (m), 1045 (m), and 750 (s) cm^{-1}; uv, λmax (EtOH) (log ϵ) 220 (4.55) and 290 (4.90) nm; pmr, δ ($CDCl_3$) 2.19 and 2.11 (each d, J=8.1 Hz, 3H, 18-CH_3), 3.64 (s, 3H, 16-CO_2CH_3), 4.48 and 4.24 (each d, J=10 Hz, 1H, 3-H), 6.68 and 6.56 (each q, J=8.1 Hz, 1H, 19-H), 7.50–7.10 (m, 4H, 9, 10, 11, 12-H), 7.76 and 7.68 (each s, 1H, 17-H), 8.02 and 7.98 (each s, 1H, indole NH), 10.29 and 9.37 (each s, 1H, 21-H); ms, *m/e* (%) M^- 350 (40), 322 (30), 321 (10), 319 (11), 318 (11), 307 (24), 291 (38), 280 (14), 279 (59), 265 (26), 264 (20), 263 (65), 249 (16), 247 (12), 223 (14), 222 (11), 221 (54), 219 (10), 209 (22), 208 (17), 184 (11), 170 (16), 169 (20), 168 (13), 167 (12), 156 (14), 155 (11), 154 (16), 144 (13), 143 (10), 130 (11), 129 (15), 128 (17), and 115 (14). These physical data are in accord with those reported for vallesiachotamine (**1**) (13).

Fractions 12–15 from the column, when combined and taken to dryness *in vacuo*, yielded a solid which on crystallization from methanol afforded needles (11.9 mg), mp 242°; uv, λmax (MeOH) (log ϵ) 228 (4.51), and 275 (3.74) nm; pmr, δ (CD_3COCD_3) 1.65 (d, J=7 Hz, 3H, 18-CH_3), 2.92 (s, 3H, 17-CO_2CH_3), 5.35 (q, J=7 Hz, 1H, 19-H), and 7.38–6.94 (m, 4H, 9, 10, 11, 12-H); ms, *m/e* (%) M^+ 352 (79), 351 (45), 337 (15), 335 (10), 322 (10), 321 (46), 293 (17), 250 (19), 249 (77), 238 (18), 235 (12), 221 (11), 182 (17), 170 (14), 169 (79), 168 (57), 167 (14), 156 (16), 154 (15), 143 (10), 130 (11), 129 (12), 128 (12), 126 (13), and 115 (19). These physical data are in agreement with those reported for polyneuridine (**2**) (14).

Characterization of sewarine (**3**).—Fraction II-G (2.3 g) was chromatographed over a column containing silica gel (50 g) packed in chloroform. A total of 15 fractions was collected and combined on the basis of their tlc patterns. Fraction 10 from the column was taken to dryness *in vacuo*. Trituration with a little methanol gave an amorphous solid (10.7 mg), mp 241–2°; ir, νmax (KBr) 3360 (s, NH and OH), 2940 (w), 2860 (w), 1660 (s), 1600 (s), 1480 (s), 1440 (m), 1200 (s), 1180 (s), 1100 (m), 1050 (m), 870 (w), 840 (w), 830 (w), and 760 (m) cm^{-1}; uv, λmax (MeOH) (log ϵ) 220 (4.0), 311 (2.3) and 340 (3.0) nm, λmax (MeOH+NaOH) 230, 318 and 358 nm; pmr, δ (2% DCl-D_2O) 1.54 (d, J=6 Hz, 3H, 18-CH_3), 3.78 (s, 3H, 16-CO_2CH_3), 5.74 (m, 1H, 19-H), 6.73 (d, J=8 Hz, 1H, 12-H), 6.82 (d, J=8 Hz, 1H, 11-H), and 6.94 (br s, 1H, 9-H); ms, *m/e* (%) M^+ 338 (90), 323 (12), 307 (15), 305 (10), 279 (22), 268 (16), 263 (18), 251 (11), 250 (15), 236 (19), 232 (11), 224 (11), 223 (16), 222 (23), 210 (10), 209 (18), 208 (14), 199 (20), 197 (11), 196 (15), 183 (15), 172 (11), 170 (19), 169 (10), 121 (100), 107 (18), 106 (21) and 92 (21). These physical data are in accord with those reported for sewarine (**3**) (15).

Characterization of tetrahydrosecamine (**4**).—Fraction V (43.7 g) was chromatographed over a column of silica gel (1.5 kg) packed in chloroform. A total of 50 fractions (500 ml each) was collected as the solvent was progressively changed to increasingly polar mixtures of benzene-chloroform, chloroform and chloroform-methanol. Fraction 13 (8.0 g) obtained from the above column by elution with chloroform was rechromatographed on a column of silica gel (400 g). Elution with chloroform yielded a crude solid which, on precipitation from ethyl acetate, afforded tetrahydrosecamine (1.1 g), ir, νmax (KBr) 3420 (br, NH), 2950 (m), 1735 (s, CO), 1460 (s), 1335 (w), 1200 (m), 1180 (m), and 750 (s) cm^{-1}; uv, λmax (EtOH) (log ϵ), 225 (5.83), 272 (5.18), 285 (5.23) and 294 (5.18)nm; pmr, δ ($CDCl_3$) 1.25–0.86 (m, 6H, CH_3), 3.34–1.97 (m, 35H, CH_2 and CH), 3.83 (s, 3H, CO_2CH_3), 3.67 (s, 3H, CO_2CH_3), 7.86–6.30 (m, 8H, aromatic) and 9.65 (br s, 1H, indole NH); ms, *m/e* (%) 680 (1.5), 126 (100). These physical data are in agreement with those of tetrahydrosecamine (**4**) (16,17).

$LiAlH_4$ reduction of tetrahydrosecamine (**4**).—Tetrahydrosecamine (**4**, 70 mg) was treated with $LiAlH_4$ (500 mg) in dry diethyl ether (15 ml) under reflux for 6 hr. Standard work-up procedures afforded a gummy mass (**5**, 18.1 mg), ir, νmax (KBr) 3400 (br NH and OH), 2940 (s), 1450 (s), 1330 (w), 1160 (w), 1050 (w) and 740 (s) cm^{-1}; uv, λmax (EtOH) (log ϵ) 230

[2]E. Merck, Darmstadt, W. Germany.

(4.78), 284 (4.20) and 290 (4.17) nm; pmr, δ ($CDCl_3$) 1.25–0.85 (m, 6H, CH_3), 3.1–2.0 (m, 37H, CH_2 and CH), 4.40 (m, 4H, C*H* and OH) and 7.50–6.70 (m, 8H, aromatic).

ACETYLATION OF TETRAHYDROSECAMINE DIOL (**5**).—A solution of tetrahydrosecamine diol (**5**, 15.0 mg) in pyridine-acetic anhydride (3 ml, 1:2) was kept at room temperature over night. The reaction mixture, when poured into cold water and extracted with chloroform (3 x 10 ml), dried over Na_2SO_4 and concentrated, gave tetrahydrosecamine diol diacetate (**6**, 12.0 mg), ir, νmax (KBr) 3400 (br NH), 2940 (s), 1745 (m, CO) 1710 (m, CO), 1470 (m), 1380 (w), 1330 (w), 1040 (w) and 750 (w) cm^{-1}; uv, λmax (EtOH) (log ε) 224 (4.77), 286 (4.16) and 292 (4.11) nm; pmr, δ ($CDCl_3$) 1.25–0.89 (m, 6H, CH_3), 2.01 (s, 3H, $OCOCH_3$, 2.06 (s, 3H, $OCOCH_3$), 3.27–2.63 (m, 35H, CH_2 and CH), 4.79 (br s, 2H, CH_2OCOCH_3), 5.44 (br s, 2H, CH_2OCOCH_3) and 7.57–6.87 (m, 8H, aromatic).

DIDEMETHOXYCARBONYLATION OF TETRAHYDROSECAMINE (**4**).—To a solution of tetrahydrosecamine (**4**, 110 mg) in ethanol (2 ml) a saturated solution of sodium hydroxide (5 ml) was added; the mixture was refluxed for 4 hr. Hydrochloric acid (5N) was added to acidify the solution which was then refluxed for 24 hr. The reaction mixture was poured into ice cold water and rendered neutral by the addition of a saturated solution of sodium bicarbonate. The solution, when extracted with chloroform (3 x 100 ml), washed with water, dried over Na_2SO_4 and concentrated, afforded a gummy mass (76.1 mg) of didemethoxycarbonyl-tetrahydrosecamine (**7**), ir, νmax (KBr) 2900 (w), 1640 (m), 1450 (m), 960 (w) and 720 (s) cm^{-1}; uv, λmax (EtOH) 228 (5.22), 286 (4.55) and 292 (4.52) nm; pmr, δ ($CDCl_3$) 1.25–0.93 (m, 6H, CH_3), 2.97–1.73 (m, 37H, CH_2 and CH), 7.67–6.75 (m, 8H, aromatic) and 9.60 (br s, 1H, indole NH).

DISCUSSION

Rhazya stricta roots and leaves of Indian origin were studied in this investigation. The total crude alkaloids (0.6%) were separated into seven fractions, and three of these were found to be cytotoxic against Eagle's KB carcinoma of the nasopharynx in cell culture. Tetrahydrosecamine (**4**, 0.0067%), a dimeric indole alkaloid previously reported from *R. stricta* leaves (16) was isolated from Fraction V by column chromatography and was shown to be responsible, at least in part, for the cytotoxicity of this fraction. Additional alkaloids could not be isolated from other chromatographic cuts of this fraction, each of which was extremely complex.

Fraction II was subjected to a gradient pH separation, vallesiachotamine (**1**, 2.6 x 10^{-6}%) and polyneuridine (**2**, 2.6 x 10^{-6}%) were isolated from the pH 6.7 fraction; sewarine (**3**, 2.4 x 10^{-6}%) was obtained from the pH 9.0 fraction. The remaining pH fractions of Fraction II were complex and did not yield alkaloids by conventional techniques.

TABLE 3. Cytotoxic activity of alkaloids of *Rhazya stricta*

Alkaloid	*in vitro*	
	KB results[a]	P-388 results[a]
	ED_{50} μg/ml	ED_{50} μg/ml
1. Vallesiachotamine	3.56	1.1
2. Polyneuridine	>100	>100
3. Sewarine	—	2.7
4. Tetrahydrosecamine	0.69	0.40
5. Tetrahydrosecamine-diol	0.0038	0.33
6. Tetrahydrosecamine-diol diacetate	2.40	1.60
7. Didemethoxycarbonyl tetrahydrosecamine	0.50	0.29

[a]A compound is considered active if it displays an $ED_{50} \leqq 4.0$ μ (12).

Tetrahydrosecamine (**4**) was reduced by LAH/ether to spectrum confirmed the absence of carbonyl absorption; this w by the absence of characteristic signals at δ 3.83 and 3.67 fo signals in the pmr spectrum of tetrahydrosecamine.

Treatment of tetrahydrosecamine diol (**5**) with acetic

for 24 hr. yielded a gummy mass, shown to be its diacetate (**6**) by spectral data. It exhibited absorptions at 223, 284, 292 nm in its uv spectrum, while in the ir spectrum the bands at 1745 and 1710 cm^{-1} were indicative of two ester carbonyls. The pmr spectrum showed two singlets, each integrating for three protons at δ 2.06 and 2.01 attributable to two acetylmethyl protons.

Spectroscopic examination of the product from the treatment of tetrahydrosecamine (**4**) with ethanolic alkali and subsequent heating with 5N hydrochloric acid at 100° for 24 hr. indicated the presence of didemethoxycarbonyl-tetrahydrosecamine (**7**).

Polyneuridine, previously isolated from *Aspidosperma polyneuron* (11), was observed in *R. stricta* leaves for the first time.

Biological activity of the isolates.—The anticancer activity data for the isolated alkaloids of *R. stricta* described here are summarized in table 3. Tetrahydrosecamine diol has surprisingly good activity in the KB carcinoma of nasopharynx test system *in vitro*, and additional studies of related derivatives are underway.

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