

## (-)- $\delta$ -N-NORMETHYLSKYTANTHINE FROM *TECOMA AREQUIPENSIS*

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**ABSTRACT.**—Bark of *Tecoma arequipensis* yielded the major alkaloidal component (-)- $\delta$ -N-normethylskytanthine (**1**) whose structure was proven by X-ray diffraction analysis of the N-thiourea derivative. The stereochemistry of **1** at all four centers was enantiomeric with that of skytanthine previously reported from *Tecoma stans* and *Skytanthus acutus*. Gc-ms of the crude base fraction indicated the presence of several related alkaloids as very minor constituents.

The genus *Tecoma* Juss. (Bignoniaceae) contains 16 species, and these shrubs or small trees are distributed throughout the tropics and subtropics. Plant extracts of *Tecoma* have often been used in folk medicine (1) particularly as hypoglycemics (2). In early work, a variety of monoterpene alkaloids were isolated, with most studies being done on *Tecoma stans*. Similar alkaloids were reported from *Skytanthus* species. Structures were reviewed (1), and no alkaloid isolations have been reported since the review, although iridoid glucosides were found in several *Tecoma* species (3). We report here on the alkaloid content of *Tecoma arequipensis* (Sprague) Sandw., a small tree native to southern Peru.

### RESULTS AND DISCUSSION

We were unable to locate *T. arequipensis* in a previously known habitat in the lower Colcha River valley of southern Peru. One tree from this area had, however, been earlier transplanted by R. Ferreyra into the botanical garden of the Museo de Historia Naturales in Lima. Wood and green bark of branches were essentially negative for alkaloids by a preliminary tlc color test. A leaf test was equivocal, and alkaloid traces may have been present. Mature bark, however, gave a very strong positive alkaloid test. One branch was cut; the bark was stripped and then dried for later analysis.

The dried bark was extracted with MeOH, and the solution was examined by tlc. Two major alkaloid spots were evident at  $R_f$  0.05 and 0.5. Trituration with  $\text{CHCl}_3$  of the residue remaining after removal of the MeOH and subsequent chromatographic separation yielded the two alkaloids, which proved to have very similar  $^1\text{H}$ -nmr spectra. Treatment of the lower  $R_f$  component with base and extraction into  $\text{CHCl}_3$  converted it into the higher  $R_f$  component. If the original MeOH extract was purified by a differential pH treatment to yield a crude base fraction, there was only one major alkaloid, identical with the higher  $R_f$  component. The pure higher  $R_f$  component was obtained by chromatography. The  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra suggested that the alkaloid was an N-normethylskytanthine. A crystalline N-4-bromophenylthiourea derivative was prepared directly from crude base fraction, and its structure, including absolute configuration, was established by X-ray diffraction as **1**. Because the optical rotation of the alkaloid was  $-21.5^\circ$ , it can be designated as (-)-N-normethyl- $\delta$ -skytanthine.

The original crude base fraction was also examined by gc-ms. By far the major peak was that for **1**. The molecular ions and fragmentation patterns of minor components suggested the presence of 5-dehydroskytanthine, skytanthine, actinidine, 5-hydroxyskytanthine, and tecomanine, all alkaloids previously known from *T. stans*. Ms data for



## EXPERIMENTAL

PLANT MATERIAL.—The specimen of *T. arequipensis* from the Colcha River valley of southern Peru was collected, grown, and identified by Dr. Ramon Ferreyra, Botanical Garden of the Museo de Historia Naturales, Arenales Av., Lima Peru. Voucher specimens and the living tree are maintained in the Botanical Garden.

ISOLATION OF (-)-N-NORMETHYL- $\delta$ -SKYTANTHINE [1].—*Method A.*—Bark (39.8 g) was ground to a powder and extracted with MeOH three times for 24 h each at room temperature. The extracts were combined and evaporated in vacuo to leave 1.88 g of residue. This was triturated with CHCl<sub>3</sub>. The CHCl<sub>3</sub> solution showed two major spots by tlc (Si gel; CHCl<sub>3</sub>-MeOH, 1:1) at  $R_f$  0.5 and 0.05. The triturate was separated into 49 fractions by Si gel flash chromatography (CHCl<sub>3</sub>-MeOH, 1:1, followed by the same solvent with 1% added NH<sub>4</sub>OH). Fractions 6–16 were combined to yield 1.25 g and fractions 40–49 to give 73 mg of crude alkaloids. These fraction combinations were further purified by flash chromatography on basic alumina, recombination of fractions, and a final acid-base partition to yield 11 mg of pure 1 from the original fractions 6–16 and 27 mg of pure 1 from the original fractions 40–49.

*Method B.*—Ground bark (8.0 g) was extracted with MeOH for 24 h (Soxhlet). Removal of the solvent in vacuo yielded 1.27 g of residue. This was dissolved in 1 M H<sub>2</sub>SO<sub>4</sub> and extracted with CHCl<sub>3</sub>; the aqueous layer was made basic to pH 9 with NH<sub>4</sub>OH and then extracted three times with CHCl<sub>3</sub>. The CHCl<sub>3</sub> was evaporated to yield 135 mg of crude alkaloid (1.7%) that showed a predominant major spot on tlc at  $R_f$  0.5, along with trace spots at other  $R_f$  values.

TABLE 1. Details of the Crystallographic Experiment and Computations for (-)-N-(4-Bromophenylthiourea)-normethyl- $\delta$ -skytanthine.

|  |  |
|--|--|
| Molecular formula . . . . .                        | C <sub>17</sub> H <sub>23</sub> N <sub>2</sub> SBr |
| Formula wt . . . . .                               | 366.96   |
| Crystal system . . . . .                           | Orthorhombic                                       |
| Space group . . . . .                              | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>      |
| Lattice constants                                  |  |
| <i>a</i> , Å . . . . .                             | 5.814 (1)  |
| <i>b</i> , Å . . . . .                             | 11.194 (3)   |
| <i>c</i> , Å . . . . .                             | 25.633 (5)   |
| $\alpha$ , deg . . . . .                           | 90   |
| $\beta$ , deg . . . . .                            | 90   |
| $\gamma$ , deg . . . . .                           | 90   |
| <i>V</i> , Å <sup>3</sup> . . . . .                | 1668.35  |
| Temperature . . . . .                              | 65° F  |
| Z . . . . .  | 4  |
| $\rho$ (observed, g cm <sup>-3</sup> ) . . . . .   | —  |
| $\rho$ (calculated, g cm <sup>-3</sup> ) . . . . . | 1.46   |
| Crystal dimensions . . . . .                       | 0.02 × 0.30 × 0.20                                 |
| Radiation . . . . .                                | MoK $\alpha$ ( $\lambda$ = 0.71073 Å)              |
| Monochromator . . . . .                            | graphite   |
| $\mu$ , cm <sup>-1</sup> . . . . .                 | 25.55  |
| Scan type . . . . .                                | $\theta/2\theta$                                   |
| Geometry . . . . .                                 | Bisecting  |
| Scan speed, deg min <sup>-1</sup> . . . . .        | 1°–30°/min   |
| 2 $\theta$ range, deg . . . . .                    | 4°–45°   |
| Index restrictions . . . . .                       | +7, +13, +28                                       |
| Total no. of reflections . . . . .                 | 1333   |
| No. or unique observed reflections . . . . .       | 1096   |
| Observed reflection criterion . . . . .            | $I > 3.5 \sigma (I)$                               |
| Final data/parameter ratio . . . . .               | 5.4  |
| R . . . . .  | 0.0399   |
| R <sub>w</sub> . . . . .                           | 0.0309   |
| GOF . . . . .                                      | 1.937  |
| <i>g</i> . . . . .                                 | $2 \times 10^{-5}$                                 |
| Slope, normal probability plot . . . . .           | 1.525  |

Purified (-)-*N*-normethyl- $\delta$ -skytanthine was an oil,  $R_f$  0.50 (Si gel; cyclohexane-NHEt<sub>2</sub>, 7:3, [ $\alpha$ ]<sub>D</sub><sup>24</sup> -21.5 ( $c$  = 7.7, CHCl<sub>3</sub>); eims  $m/z$  153 (80), 152 (56), 138 (65), 122 (55), 110 (44), 107 (33), 96 (56), 81 (34), 70 (44), 69 (28), 68 (46), 67 (38), 58 (22), 57 (14), 56 (24), 55 (30), 44 (100), 43 (52), 42 (37), 41 (61); cims NH<sub>3</sub>  $m/z$  [M + 1]<sup>+</sup> 154; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 360 MHz)  $\delta$  0.83 (d, 6.9, 3H), 0.94 (d, 7.0, 3H), 1.13 (m, 1H), 1.54 (m, 2H), 1.62 (m, 2H), 1.9 (m, 2H), 2.15 (m, 1H), 2.23 (t, 12.3, 1H), 2.34 (t, 12.3, 1H), 2.68 (dd, 4.6, 12.6, 1H), 2.76 (dd, 5.9, 12.7, 1H). <sup>13</sup>C nmr (CDCl<sub>3</sub>, 67 MHz)  $\delta$  17.43 (q), 22.29 (t), 22.40 (q), 31.49 (t), 32.07 (d), 36.14 (d), 41.53 (d), 47.30 (d), 47.77 (t), 48.56 (t); ir (NaCl, neat) 3350, 1457, 1420, 1375, 1271, 1119, 1076.

PREPARATION OF THE 4-BROMOPHENYLTHIOUREA DERIVATIVE OF 1.—Part of a crude alkaloid fraction (36 mg, method B) and 84 mg of 4-bromophenylisothiocyanate (Aldrich Chemical Co.) were heated together over a small flame for 3 min. The reaction mixture was chilled in ice until it solidified, was washed twice with 1.5 ml hexane, and crystallized twice from EtOH to yield (-)-*N*-(4-bromophenylthiourea)-normethyl- $\delta$ -skytanthine, [ $\alpha$ ]<sub>D</sub><sup>24</sup> = -26° ( $c$  = 2.79, CHCl<sub>3</sub>); <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz)  $\delta$  0.93 (d, 6.7, 3H), 1.02 (d, 6.5, 3H), 1.10 (m, 1H), 1.37 (m, 1H), 1.62 (m, 2H), 1.85 (m, 2H), 2.26 (m, 2H), 3.14 (t, 12.2, 1H), 3.61 (dd, 4.6, 13.5, 1H), 3.72 (dd, 4.3, 12.0, 1H), 4.09 (dd, 4.4, 13.6, 1H), 6.95 (br s, NH, 1H), 7.15 (d, 8.7, 2H), 7.44 (d, 8.7, 2H); <sup>13</sup>C nmr (CDCl<sub>3</sub>, 67 MHz)  $\delta$  16.27, 19.81, 23.93, 29.90, 33.39, 37.52, 39.93, 46.08, 47.15, 50.30, 118.23, 125.95, 131.76, 139.26, 182.08.

X-RAY DATA.<sup>1</sup>—Pertinent data are given in Tables 1 and 2. The absolute configuration of 1 was checked by refinement of a multiplicative factor on the imaginary part of the anomalous dispersion connection term. Refinement of that parameter converged at 1.12 (4), which indicated that the absolute configuration shown in Figure 1 was correct.

GC-MS OF THE CRUDE ALKALOID FRACTION.—A sample of the crude alkaloid fraction from isola-

TABLE 2. Atomic Coordinates ( $\times 10^4$ ) and Isotopic Thermal Parameters ( $A^2 \times 10^3$ )<sup>a</sup> for (-)-*N*-(4-Bromophenylthiourea)-normethyl- $\delta$ -skytanthine.

| Atom           | x         | y        | z       | U iso <sup>b</sup> |
|----------------|-----------|----------|---------|--------------------|
| Br . . . . .   | 1283(1)   | -101(1)  | 805(1)  | 61(1)*             |
| S . . . . .    | 7871(4)   | 1916(2)  | 2839(1) | 57(1)*             |
| N-1 . . . . .  | 10159(8)  | 152(4)   | 3307(2) | 34(2)*             |
| N-2 . . . . .  | 7730(12)  | -419(6)  | 2652(2) | 50(3)*             |
| C-1 . . . . .  | 10907(14) | -1038(5) | 3365(2) | 43(3)*             |
| C-2 . . . . .  | 12817(12) | -1214(6) | 3770(2) | 47(3)*             |
| C-3 . . . . .  | 12009(11) | -721(6)  | 4302(2) | 44(3)*             |
| C-4 . . . . .  | 10233(12) | -1492(5) | 4594(3) | 53(3)*             |
| C-5 . . . . .  | 8581(13)  | -594(5)  | 4839(2) | 46(2)*             |
| C-6 . . . . .  | 8420(11)  | 387(5)   | 4435(2) | 35(2)*             |
| C-7 . . . . .  | 10924(11) | 522(5)   | 4244(2) | 36(2)*             |
| C-9 . . . . .  | 7322(14)  | 1547(6)  | 4632(3) | 61(3)*             |
| C-10 . . . . . | 13654(16) | -2504(5) | 3798(3) | 68(3)*             |
| C-8 . . . . .  | 11090(14) | 1013(5)  | 3688(2) | 45(2)*             |
| C-11 . . . . . | 8618(12)  | 505(5)   | 2951(2) | 36(2)*             |
| C-12 . . . . . | 6197(11)  | -325(5)  | 2228(2) | 35(2)*             |
| C-13 . . . . . | 4114(10)  | 287(5)   | 2266(2) | 45(2)*             |
| C-14 . . . . . | 2657(11)  | 347(5)   | 1854(2) | 41(2)*             |
| C-15 . . . . . | 3240(10)  | -228(5)  | 1386(2) | 38(2)*             |
| C-16 . . . . . | 5245(11)  | -837(5)  | 1345(3) | 44(2)*             |
| C-17 . . . . . | 6724(11)  | -897(5)  | 1764(2) | 42(3)*             |

<sup>a</sup>Estimated standard deviations in the least significant digits are given in parentheses.

<sup>b</sup>For values with asterisks, the equivalent isotropic U is defined as 1/3 of the trace of the U<sub>ij</sub> tensor.

<sup>1</sup>Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.

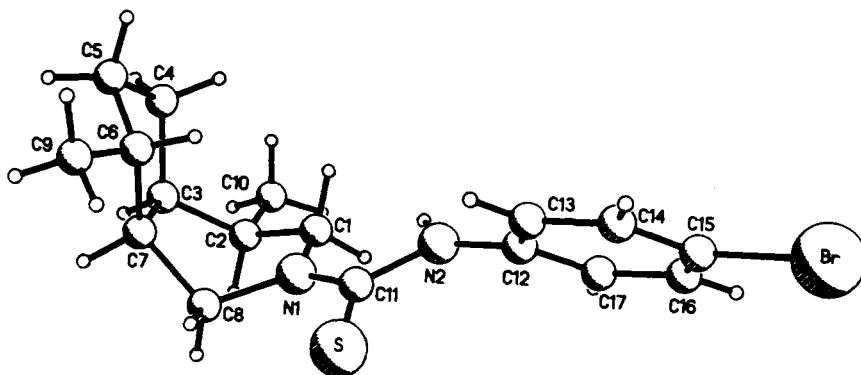


FIGURE 1. Structure of (-)-N-(4-bromophenylthiourea)-normethyl- $\delta$ -skytanthine.

tion method B was evaporated onto the end of a dropping needle injector for injection onto a 30-m J&W DB-1 methylsilicone column: injection port at 250°, and 100° column temperature for 2 min, followed by a 5°/min increase. The gc was connected to a VG Micromass 16F spectrometer. Pertinent peaks were as follows [alkaloid structure from molecular ion and fragmentation pattern (10), retention time, *m/z* (rel. int.)]: a dehydroskytanthine, 12.0 min, 165 (11), 164 (7), 93 (4), 91 (4), 79 (4), 77 (3), 58 (50), 57 (7), 44 (38), 32 (64), 28 (100); a skytanthine, 12.5 min, 167 (46), 166 (100), 152 (16), 122 (10), 110 (11), 84 (18), 58 (98), 57 (16), 44 (100), 43 (25), 42 (18), 41 (18), 40 (35); **1**, 13 min, see above; an actinidine, 16 min, 147 (51), 146 (30), 132 (100), 131 (17), 117 (33), 103 (4), 91 (4), 77 (7), 55 (1), 44 (81); a hydroxyskytanthine, 17 min, 183 (44), 182 (58), 166 (25), 165 (36), 164 (25), 150 (36), 148 (22), 132 (13), 122 (30), 107 (52), 106 (3), 93 (20), 91 (16), 84 (64), 74 (33), 58 (100), 57 (46), 44 (100), 43 (37), 42 (47), 41 (30), a tecomanine, 20.5 min, 179 (53), 164 (15), 150 (5), 136 (11), 134 (8), 121 (16), 111 (20), 108 (7), 105 (9), 93 (23), 91 (17), 57 (100), 44 (76), 42 (36), a tecomanine, 21 min, 179 (38), 178 (54), 164 (18), 151 (71), 136 (100), 122 (18), 108 (26), 94 (15), 93 (22), 91 (17), 79 (15), 77 (12), 58 (15), 53 (16), 44 (100), 42 (37), 41 (18), 40 (19), a tecomanine, 22.5 min, 179 (46), 164 (14), 150 (6), 136 (15), 121 (15), 111 (19), 93 (29), 91 (25), 79 (10), 77 (10), 58 (30), 57 (100), 44 (100), 42 (47), 40 (25), a hydroxytecomanine, 23 min, 195 (23), 180 (12), 152 (10), 138 (9), 123 (12), 112 (13), 109 (13), 91 (10), 81 (14), 79 (9), 77 (9), 58 (100), 57 (43), 44 (100), 43 (35), 42 (40), 40 (25), a dihydrotecomanine, 24.7 min, 181 (100), 166 (36), 152 (29), 138 (35), 122 (82), 109 (92), 107 (63), 95 (26), 93 (20), 81 (63), 73 (67), 72 (40), 67 (34), 58 (24), 55 (34), 53 (20), 44 (85), 43 (27), 42 (36), 41 (61), a hydroxytecomanine, 26 min, 195 (100), 180 (44), 166 (21), 152 (61), 138 (39), 124 (29), 122 (40), 109 (52), 107 (35), 96 (35), 87 (79), 81 (36), 72 (25), 70 (26), 55 (25), 44 (100), 43 (66), 42 (24), 41 (41).

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