THE ALKALOIDS OF STEPHANIA GLABRA

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From the tubers of Stephania glabra (Roxb) Miers (synonym rotunda), have been isolated rotundine [1, 2], *l*-tetrahydropalmatine, and hindaricine [3, 4]. We have investigated the alkaloids contained in the tubers of Stephania glabra obtained from India and also in the tubers and epigeal parts of the plant cultivated on the Black Sea littoral of the Caucasus.

Two alkaloids were isolated from the tubers of Indian origin. The first of them had a composition, and constants of the salts and the base, agreeing with the composition and constants of *l*-tetrahydropalmatine. To confirm the identity of these substances, we converted the *l*-tetrahydropalmatine into palmatine [4, 5]. This gave a compound identified by the melting points of the iodide and the perchlorate, by a mixed melting point of the perchlorates, and by the IR spectra, as palmatine.

The second alkaloid had the composition $C_{18}H_{19}O_3N$, mp 182-183°, $[\alpha]^{21}_D$ +144° (chloroform). The three oxygen atoms present in the molecule of this substance are distributed between two methoxy groups and a carbonyl group conjugated with a double bond, as is shown by the absorption band of a conjugated carbonyl group in the IR spectrum (1670 cm^{-1}); UV spectrum: λ_{max} 232, 282 mμ (log ε 4.194; 3.446). The alkaloid contains a secondary amino group, as follows from the possibility of obtaining a N-acetyl derivative of empirical formula $C_{20}H_{21}O_4N$. The IR spectrum of the latter has the bands of an amide group (1634 cm^{-1}) and a conjugated carbonyl group (1670 cm^{-1}); there is no absorption, characteristic of NH or OH groups. Reduction of the N-acetate with sodium borohydride gave a dihydro derivative the IR spectrum of which retained the amide carbonyl band (1634 cm^{-1}), lacked the band of the conjugated ketone, and had the band of a hydroxyl group (3200 cm^{-1}). With respect to all the constants that we determined, this alkaloid proved to be identical with the base stepharine isolated by American workers from S. glabra [6] almost simultaneously with our alkaloids.*

From herbage of S. glabra of the same origin, we isolated cycleanine and a base of the composition $C_{19}H_{21}O_4N$, mp 205-206°; $[\alpha]^{21}_D$ −70°, which we have provisionally called "base A". The substance contains two OCH_3 groups. The IR spectrum contains absorption bands at 3300 (OH group) and 1670 cm^{-1} (conjugated carbonyl group). The solubility of the compound in alkalis and the positive ferric chloride reaction show the phenolic nature of the hydroxyl group. UV spectrum: λ_{max} 242, 282 mμ (log ε 4.182, 3.603). A hydrogenation derivative with the empirical formula $C_{19}H_{27}O_4N$, mp 210-211° has been obtained. The IR spectrum of this compound no longer contains the absorption band of the conjugated carbonyl group. These results suggest that base A may be N-methylhydroxystepharine.

Experimental

Isolation of *l*-tetrahydropalmatine from the tubers of S. glabra. The alkaloids were extracted from 1 kg of the air-dry ground tubers by the usual method with xylene. The extract was treated twice with 10% sulfuric acid. The combined sulfuric acid solutions were made alkaline with 25% aqueous ammonia. The precipitate which was deposited (36 g) was washed with water and, after drying in a vacuum desiccator, was suspended in 360 ml of alcohol; the suspension was treated with a saturated solution of hydrogen chloride in alcohol until it was acid to congo red. After some hours, *l*-tetrahydropalmatine hydrochloride with mp 257° (from alcohol) was precipitated, yield 9.6 g. The alcoholic mother liquor was evaporated to 1/8 of its initial volume and the concentrate obtained was added to the aqueous mother solution remaining after the removal of the *l*-tetrahydropalmatine base. The mixture was made alkaline with concentrated ammonia, after which the alkaloids were extracted exhaustively with chloroform. The chloroform solution was treated with 10% sulfuric acid. Stepharine (fraction A) was found in the sulfuric acid solution. After the stepharine had been removed, the chloroform extract was washed with 25% ammonia solution and was dried over sodium sulfate. The residue after the distillation of the chloroform was dissolved in 130 ml of alcohol and a saturated alcoholic solution of hydrogen chloride was added until an acid reaction was obtained. An additional amount of *l*-tetrahydropalmatine hydrochloride (10.5 g) was obtained. From this the base was obtained in the usual way; mp 141-142° (from alcohol), $[\alpha]^{21}_D$ −828° (c 0.5; chloroform).

Found, %: C 71.19, 71.33; H 7.14, 7.21; N 3.98, 3.99. Calculated for $C_{21}H_{25}O_4N$, %: C 70.98; H 7.04; N 3.94.

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*We filed an application (No. 81770/31-16) on the alkaloid with the Committee for Inventions and Discoveries of the Council of Ministers of the USSR on 4 February 1963, but it was rejected because of the absence of results of clinical tests.

l-Tetrahydropalmatine sulfate was obtained by mixing alcoholic solutions of the base and sulfuric acid, mp 218° (from alcohol).

l-Tetrahydropalmatine methiodide was isolated by boiling an alcoholic solution of *l*-tetrahydropalmatine (0.5 g) and methyl iodide (1 g) for 15 min. The reaction product melted at 257° (from alcohol).

Palmatine iodide. A solution of 0.5 g of *l*-tetrahydropalmatine in 10 ml of hot alcohol was treated with 0.5 g of iodine in 25 ml of alcohol and the mixture was boiled for 2 hr; a brown precipitate (0.6 g) was deposited which was suspended in hot water. To eliminate the excess of iodine, sulfur dioxide was passed through the suspension; after filtration and subsequent crystallization from hot water and then from 85% alcohol, the iodide, with mp 241°, was obtained.

Found, %: C 52.85, 53.00; H 4.81, 4.76; N 2.83, 2.90; I 26.39, 26.56. Calculated for $C_{21}H_{22}O_4N$, %: C 52.81; H 4.80; N 2.92; I 26.51.

A saturated solution of sodium perchlorate was added to an aqueous solution of palmatine iodide; the perchlorate which was deposited had mp 268-269° (from alcohol).

Isolation of stepharine. The sulfuric acid solution containing the stepharine (fraction A) was made alkaline with 25% ammonia solution and the base was extracted with chloroform. The extract was dried with sodium sulfate and evaporated. The syrupy residue was treated with 10% sulfuric acid until the sulfate of the alkaloid with mp 245-246° (50% alcohol) had been precipitated completely, yield 4.0 g.

To obtain stepharine, an aqueous solution of the sulfate of the alkaloid was made alkaline with ammonia and was extracted with chloroform. The extract was dried and evaporated. The residue was crystallized successively from alcohol, acetone, and benzene. The needle-like crystals had mp 182-183° (decomp), $[\alpha]^{21}_{D}$ +144° (c 0.79; chloroform). The IR spectrum had bands in the 3240-1670 cm^{-1} region.

Found, %: C 72.13, 71.91; H 6.77, 6.22; N 5.14, 4.96; OCH_3 18.68, 19.22; mol. wt. 296 (potentiometric), 287 (cryoscopic). Calculated for $C_{18}H_{19}O_3N$, %: C 72.70; H 6.39; N 4.71; OCH_3 20.08; mol. wt. 297.

Stepharine hydrochloride was deposited when alcoholic solutions of the base and hydrochloric acid were mixed, mp 253-254° (decomp).

Found, %: C 64.80, 64.58; H 6.15, 6.37; N 4.33, 4.52; Cl 10.34, 10.13. Calculated for $C_{18}H_{19}O_3N$, %: C 65.06; H 5.99; N 4.19; Cl 10.64.

When alcoholic solutions of the base and acids were mixed, the corresponding salts were obtained:

Picrate, mp 115-116°.

Found, %: C 55.15, 55.51; H 4.33, 4.54; N 10.63, 10.58. $C_{18}H_{19}O_3N$. Calculated for $C_6H_3O_7N_3$, %: C 54.75; H 4.18; N 10.64

Sulfate, mp 243-244°.

Found, %: N 3.89, 3.86; S 5.05, 5.09. Calculated, %: N 4.04; S 4.62.

Chloroplatinate, mp 210° (decomp).

N-Acetylstepharine. A solution of 0.3 g of the base in 3 ml of pyridine was treated with 1.5 ml of acetic anhydride. The mixture was left for 14 hr at room temperature. The reaction product had mp 230-231° (from alcohol). The IR spectrum had absorption bands at 1670 and 1634 cm^{-1}.

Found, %: C 70.70, 71.07; H 6.30, 6.38; N 4.40, 4.24. Calculated for $C_{20}H_{21}O_4N$, %: C 70.79; H 6.19; N 4.10.

Reduction of N-acetylstepharine. A solution of 0.08 g of N-acetylstepharine and 0.08 g of sodium borohydride in 40 ml of 50% methanol was left for 14 hr at room temperature. A substance was deposited which had mp 145-146° and bands in the IR spectrum at 3400 and 1634 cm^{-1}.

Isolation of the alkaloids from the epigeal parts. The dried and comminuted epigeal parts (10 kg) of the plant were wetted with 12% ammonia and the alkaloids were extracted exhaustively with dichloroethane. The dichloroethane extract was shaken with 10% sulfuric acid. After the acid solution had been made alkaline with 25% ammonia, the alkaloids were extracted with chloroform. The chloroform solution was dried with sodium sulfate and evaporated to dryness. This gave 108 g of total bases, which were dissolved in methanol and ether (1 : 25) and chromatographed on 5.4 kg of alumina (activity grade II). On elution with the same mixture of solvents, cycleanine (12 g) and then a mixture of cycleanine and base A (15 g) were obtained.

Cycleanine, after crystallization from acetone and then from alcohol, had mp 272-273°, $[\alpha]^{21}_{D}$ – 12° (c 0.5; chloroform).

Found, %: C 73.21, 73.45; H 6.89, 6.93; H 4.40, 4.49. Calculated for $C_{38}H_{42}O_6N_2$, %: C 73.07; N 6.73; N 4.49.

Cycleanine picrate, mp 193°.

Cycleanine methiodide melted at 310-311°.

N-Methyl-des-cycleanine was obtained by shaking 0.4 g of the methiodide with an excess of freshly-prepared silver oxide in aqueous methanol for 1.5 hr. After the removal of the silver iodide, the solution was distilled. After crystallization from alcohol, the substance obtained melted at 209-210°.

Base A. After crystallization from alcohol and acetone, the alkaloid formed colorless crystals with mp 205-206°, $[\alpha]_D^{21}$ −70° (c 1; chloroform).

Found, %: C 69.70, 69.56; H 6.55, 6.47; N 4.22, 4.30; OCH_3 18.52, 19.00; H (labile) 5.30, 6.11; mol. wt. 307 (Rast). Calculated for $C_{19}H_{21}O_4N$, %: C 69.42; H 6.40; N 4.26; OCH_3 18.96; H (labile) 5.19.

Picrate, mp 226-226.5°.

Found, %: C 53.89, 53.83; H 4.42, 4.63; N 10.03, 9.90 $C_{19}H_{21}O_4N$. Calculated for $C_6H_3O_7N_3$, %: C 53.95; H 4.32; N 10.07.

Hydrochloride, mp 179-180°.

Hydrogenation derivative of the base A. A solution of 0.4 g of the base in ethanol was hydrogenated over platinum (0.1 g); 3 moles of hydrogen was absorbed. The hydrogenation product, after crystallization from acetone and then from benzene, melted at 211-212°.

Found, %: C 68.86, 69.00; H 8.11, 8.06; N 4.33, 4.39. Calculated for $C_{19}H_{27}O_4N$, %: C 68.46; H 8.10; N 4.20.

Summary

l-Tetrahydropalmatine and stepharine have been isolated from the tubers of Stephania glabra obtained from India, and *l*-tetrahydropalmatine and cycleanine have been isolated from the tubers of Stephania cultivated on the Black Sea littoral of the Caucasus.

The epigeal parts of the plant cultivated on the Black Sea littoral of the Caucasus have given cycleanine and an alkaloid A of composition $C_{19}H_{21}O_4N$ the study of which is proceeding.

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