ALKALOIDS OF *Sophora alopecuroides*

3-α-HYDROXYSOPHORIDINE

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Continuing an investigation of the alkaloids of Sophora alopecuroides [1], we have studied the epigeal part of this plant collected by I. A. Gubanov in Central Asia (at the village of Tyul'kubas) on August 8, 1966 in the fruit-bearing stage. The combined alkaloids obtained by the usual dichloroethane method (2.5%) were separated into weakly and strongly basic fractions. The following alkaloids were extracted from the weakly basic fraction with various solvents in combination with chromatography on alumina: sophoridine, cytisine, and three unknown bases (III with the composition $C_{13}H_{18}N_2O_2$, IV with the composition $C_{15}H_{24}N_2O_2$, and VI also with the composition $C_{15}H_{24}N_2O_2$). From the strongly basic fraction we obtained sophoridine, cytisine, and baptifoline (V). This is the first time that the alkaloids cytisine and baptifoline have been isolated from this plant.

The IR spectrum of the base (III) shows absorption bands at 3500 and 3700 cm^{-1} (free and bound alcoholic hydroxyls) and 1660 cm^{-1} (α, β, γ, δ-unsaturated lactam carbonyl). The mass spectrum of this base contains peaks with m/e 234 of M^+ (9%) and 203 (100%) and also 160 (20%), 146 (14%), 117 (7%), 88 (49%) and 58 (84%). In the nature of its fragmentation it resembles the mass spectrum of methylcytisine [2] (peaks of fragments with m/e 160, 146, 117, and 58) but differs from it by the fragmentation peaks with m/e 88 and 203. The difference of 30 amu between the peaks with m/e in the mass spectrum of the base (III) and m/e 58 [$CH_2=N(CH_3)_2$] in the mass spectrum of methylcytisine shows the presence of an additional CH_2O group in the fragment with m/e 88. In addition, the peak with m/e 203 (M−31) shows the elimination of a CH_3O group. Since, according to its NMR spectrum, the base (III) has a $N-\overset{\beta}{C}H_2-\overset{\alpha}{C}H_2OH$ group (α-CH_2 3.40 ppm, t, $\varepsilon_J = 12$ Hz, 2H; β-CH_3 2.35 ppm, t, $\varepsilon_J = 12$ Hz, 2H) it must be ascribed the structure of N-hydroxyethylcytisine. Thus, this compound is an artefact and is formed from cytisine during the dichloroethane extraction.

The IR spectrum of the base (IV) (Fig. 1) shows absorption bands at 3620 cm^{-1} (free hydroxyl), 2180 cm^{-1} (trans-quinolizidine), and 1620 cm^{-1} (lactam carbonyl). The mass spectrum of this base contains the peaks 264 M^+ (78%), 263 (M−1) (100%), 205 (31%), 193 (26%), 166 (37%), M−17 (10%) and others. The presence of the M−1 peak, and also the nature of the fragmentation, makes it possible to assign the base (IV) to the alkaloids of the matrine series [4], with a different configuration at C_6 from sophoridine, for example, where M−1 likewise represents 100% [5]. Its molecular peak differs from that of sophoridine by 16 mu; i.e., it also shows the presence of a hydroxy group in it. The IR spectrum of (IV) is extremely similar to that of sophoridine, differing from it only by an additional adsorption band at 3620 cm^{-1} (OH group). Furthermore, its spectrum differs from the spectra of other known isomers of the

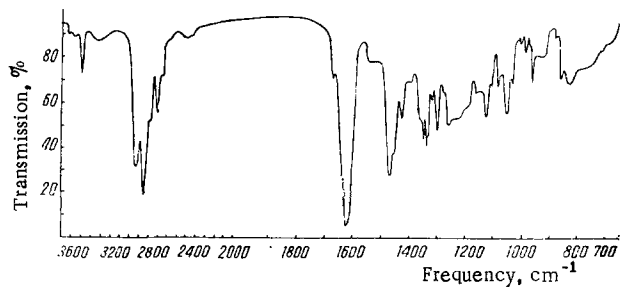

Fig. 1. IR spectrum of the base (IV).

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TABLE 1

Substance	m/e (% intensity)					
	M+	a	d	g	i	o
Matrine	248 (100)	247 (98,7)	205 (77,6)	177 (23,5)	150 (14,6)	96 (37,5)
Sophoridine	248 (36)	247 (100)	205 (25)	177 (19,3)	150 (37,5)	96 (37)
The base (IV)	264 (78)	263 (100)	205 (31).	193 (26)	166 (37)	112 (26)

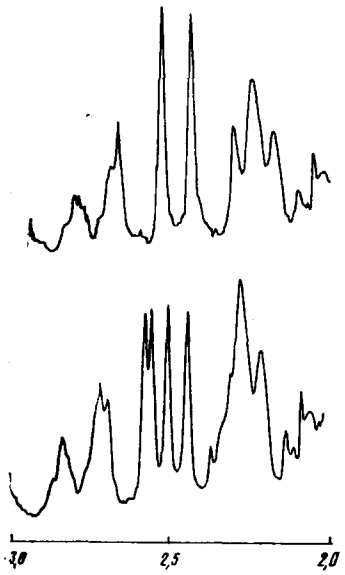
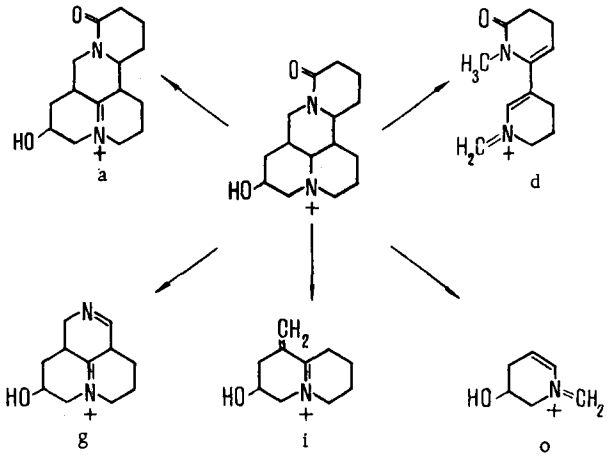


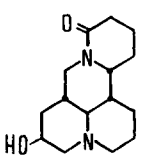
Fig. 2. Fragment of the NMR spectrum of the O-acetyl derivative on the base (IV) [the double resonance spectrum with the frequency of an additional radiofrequency field corresponding to the chemical shift of the H–C–OAc proton (4.82 ppm) is shown at the top].

matrine alkaloids [6]. The fact that the alkaloids belong to the sophoridine series is also shown by the near coincidence of the optical rotatory dispersion curves that we have obtained for these two alkaloids, which have a smooth negative character in the 600-300-nm region. On comparing the mass spectra of matrine, sophoridine [4, 5], and the base (IV) (Table 1) it can be seen that the peaks corresponding to the fragments g, i, and o containing rings A and B are shifted by 16 mass units in the case of the base (IV). Consequently, these fragments contain the hydroxy group, while, as can be seen from Table 1, the corresponding fragments of both alkaloids possess similar intensities. According to S. Iskandarov, S. Yu. Yunusov, et al. [4, 5] the ion with m/e 205 in the mass spectra of the matrine alkaloids is formed by the elimination of the C_3, C_4, and C_5 carbon atoms from rings A and C and has the structure d.



The mass spectrum of the base (IV) and that of its O-acetyl derivative [m/e 306 M^+ (21%), 305 M–1 (20%), 246 M–60 (100%), 231 (17%), 218 (22%), 205 (6%), 190 (18%), 148 (27%), 134 (22%), 96 (14%), 84 (13%), 69 (13%), 55 (24%)] also contain this ion, which permits the hydroxy group to be located in the fragment that is eliminated. However, in the mass spectrum of the acetate of the base (IV), the strongest peak is the ion with m/e 246 (M^+–60), due to the splitting out of acetic acid with the formation of a double bond in ring A, which prevents further decomposition with the formation of ion d with m/e 205, and therefore the latter has a low intensity (6%).

The position of the hydroxyl was determined from the NMR spectrum of the O-acetyl derivative of the base (IV). The signal of the proton geminal to the acetoxy group appears in the form of a multiplet with its center at 4.82 ppm. The sum of the spin–spin coupling constants (25 Hz) shows that the proton under consideration is axial and has two methylene groups in the α position. It was shown by the double resonance method (Fig. 2) that the spectral parameters of one of these methylene groups are as follows: $\delta_a = 2.43$ ppm, $\delta_e = 2.65$ ppm, $J_{gem} = 13.2$ Hz, $J_{aa} = 6.2$ Hz, $J_{ea} = 2.1$ Hz. The values of the chemical shifts show the location of this group adjacent to the nitrogen atom, and the nature of the splitting of the signals excludes the presence of additional vicinal protons. Thus, the NMR spectrum shows that the hydroxy group is located in position 3 (ring A) or 9 (ring B). However, the mass spectrum permits position 9 to be excluded; consequently, to base (IV) may be ascribed the structure of 3α-hydroxysophoridine, which agrees well with the biogenetic scheme connecting the alkaloids of the matrine and the sparteine series [7]



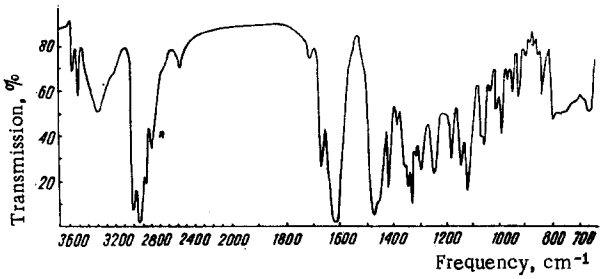


Fig. 3. IR spectrum of the base (VI).

The base (V) with mp 206-208°C, $[\alpha]_D^{20}$ −149° (c 0.243; ethanol), mol. wt. 260 (mass spectrometrically) was isolated in small amount (0.07 g). The nature of its fragmentation under electron impact shows that this compound is an alkaloid of the sparteine series [2]. In the mass spectrum of the base (V) [m/e 260−M^+ (59%), M−17 (17%), 160 (20%), 146 (40%), 114 (100%), 96 (43%), 70 (43%)] the peaks with m/e 146 and 160 coincide with the peaks of the mass spectrum of anagyrine [2], and the strongest peak with m/e 114 is shifted by 16 amu (just like M^+) as compared with the strongest peak in the mass spectrum of anagyrine, which shows the presence of a hydroxyl in ring D. The coincidence of the IR spectra of the known alkaloid baptifoline, which contains a hydroxyl in ring D, and the base (V) enabled it to be identified as baptifoline.

The base (VI), mp 214-215°C, mol. wt. 264 (mass spectrometrically). Its IR spectrum (Fig. 3) shows absorption bands at (cm^{-1}) 3630 (free hydroxyl), 3400 (bound hydroxyl), 2815 (trans-quinolizidine), and 1620 and 1670 (amide carbonyl). The mass spectra of the bases (IV) and (VI) are very similar and differ only in the intensity of the peak with m/e 193 [26% for the base (IV) and 42% for the base (VI)]. The similarity of the mass spectra of these alkaloids shows that their structures are similar.

EXPERIMENTAL

The IR spectra were taken on a UR-10 instrument, the mass spectra on an MKh-1303 at 100°C with an ionizing voltage of 706 V, and the NMR spectra on a Varian HA 100D ($CDCl_3$, 0 − HMDS). The melting points were determined on a Kofler block. The analyses of all the compounds corresponded to the calculated figures.

Isolation of the Alkaloids. The comminuted herb Sophora alopecuroides (5.5 kg) was moistened with 10% ammonia, and the alkaloids were extracted exhaustively with dichloroethane. The dichloroethane extracts were treated with 10% sulfuric acid, the acid solution was made alkaline with ammonia, and the alkaloids were extracted with chloroform, which gave 138 g (2.5%) of total alkaloids. Thin-layer chromatography on alumina [activity grade IV, petroleum ether − diethyl ether (1 : 1)−5% methanol system] showed the presence of about 14 alkaloids.

Separation of the Alkaloids. The total alkaloids (138 g) were dissolved in 5% sulfuric acid and the solution was made alkaline with sodium bicarbonate, permitting their separation into two fractions with pH 6.0 and 8.0. The fraction with pH 6 (98.7 g) was treated successively with low-boiling petroleum ether (fraction A), acetone (fraction B), and benzene (fraction C). From fraction A, 16.55 g of sophoridine with mp 208-210°C was obtained (a mixture with an authentic sample of the alkaloid gave no depression of the melting point, and their IR spectra were identical). Fraction B yielded 0.15 g of the base (VI) with mp 214-215°C (from acetone); mol. wt. 264 (mass spectrometrically). Fraction C was chromatographed on a column of alumina (activity grade IV, 1 : 50). Benzene eluted 3 g of sophoridine, and the benzene−5% chloroform eluate gave 2.5 g of cytisine with mp 151-153°C, $[\alpha]_D^{20}$ −108° (c 0.499; ethanol). A mixture with an authentic sample gave no depression of the melting point, and their IR spectra were identical. The combined residual benzene-insoluble alkaloids were chromatographed on alumina (activity grade IV, 1 : 50). The chloroform eluate yielded 2.27 g (0.042%) of N-hydroxyethylcytisine with mp 63-65°C, $[\alpha]_D^{20}$ −187° (c 0.725; ethanol) (hydriodide, mp 249-251°C). NMR spectrum (CD_3OD): C_3−H 6.33 ppm; C_4−H 7.37 ppm; C_5−H 6.20 ppm; J_{34} = 9.7 Hz; J_{35} = 14 Hz; J_{45} = 6.9 Hz.

A chloroform−5% methanol eluate yielded 1.16 g (0.021%) of 3α-hydroxysophoridine with mp 162-164°C, $[\alpha]_D^{20}$ −50.6 (c 0.555; ethanol), mol. wt. 264 (mass spectrometrically).

Acetylation of 3α-Hydroxysophoridine. A solution of 0.07 g of 3α-hydroxysophoridine in 10 ml of chloroform was treated with 0.6 ml of acetic anhydride, and the mixture was boiled under reflux for 4 h. The resinous precipitate was passed through a column containing 20 g of alumina (activity grade IV) and was eluted with a mixture of benzene and chloroform (1 : 1). The crystals of 3α-acetoxysophoridine so obtained were recrystallized from ether, mp 145-147°C. NMR spectrum (CD_3OD): 1.98 ppm (CH_3COO).

The fraction with pH 8 (32 g), consisting, according to thin-layer chromatography, mainly of cytisine, was crystallized. After the separation of the crystals and their repeated recrystallization from acetone,

6.65 g of cytisine was isolated (the total yield of cytisine being 8.9 g, 0.16%). The residual part was separated on a column of alumina (activity grade IV, 1:50). The benzene eluate yielded 0.6 g of sophoridine (total yield 20.15 g, 0.37%) and the benzene–5% chloroform eluate, 0.07 g of baptifoline with mp 206-208°C, $[\alpha]_D^{20}$ –149° (c 0.263; ethanol).

SUMMARY

The epigeal part of Sophora alopecuroides has given 2.5% of combined alkaloids, from which sophoridine (0.37%), cytisine (0.16%), N-hydroxyethylcytisine (0.042%), baptifoline (0.0013%), and the new bases (IV) (0.021%) and (VI) (0.0027%) have been isolated; the structure of 3α-hydroxysophoridine has been proposed for (IV).

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