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ALKALOIDS OF NUPHAR LUTEUM

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The alkaloids of Nuphar luteum L. (European cowlily) have been studied by the Polish scientists Achmatowicz, et al., [1] since 1939. From the dry rhizomes, they isolated the liquid bases α- and β-nupharidines with the composition $C_{15}H_{23}ON$. The structure of these compounds was established by Arata and Ohashi [2]. The alkaloid α-nupharidine is a derivative of furanoquinolizidine identical with the deoxynupharidine from Japanese Nuphar. This structure was confirmed by Kaneko, Kawasaki, and Okamoto [3] by the complete synthesis of dl-deoxynupharidine. The absolute configuration of this alkaloid has also been established [4].

We have investigated the composition of the alkaloids from the dry rhizomes of Nuphar luteum collected in the Ukraine and in Krasnodarsk Krai. The total alkaloids obtained by the usual dichloroethane method from different parts of the raw material amounted to 0.3–0.8% of the air-dry plant substance. The total alkaloids were freed from basic resins by chromatography on alumina and were separated into three fractions. Only three alkaloids were found, with R_f 0.79, 0.68, and 0.59 (in paper chromatography with 1-butanol saturated with 5% acetic acid.)

Properties	**Thiobinupharidine**	**Neothiobinupharidine**	**Base**	
			R_f 0.68	R_f 0.59
Composition of the base	$C_{30}H_{42}O_2N_2S$	$C_{30}H_{42}O_2N_2S_2$	$C_{30}H_{42}O_2N_2S$	$C_{30}H_{42}O_2N_2S$
Mp, °C	129—130	159—160	129—130	158—159
$[\alpha]_D$, deg	+49.4	—	+48.8 (c 1:1; ethanol)	
M	494	494	494 (mass spectrometrically)	498 (cryoscopically)
Perchlorate, mp, °C	282—284	320 (decomp.)	282—283	315—320 (decomp.)

From the first fraction were isolated crystalline bases (R_f 0.68 and 0.59) with mp 129°–130° C and 158°–159° C, the composition of which corresponded to the formula $C_{30}H_{42}O_2N_2S$. At the same time as we, Achmatowicz [5, 6] isolated and characterized alkaloids of the same composition, calling them thiobinupharidine (I) and neothiobinupharidine, respectively. The constants of our alkaloids, thiobinupharidine and neothiobinupharidine, are given in the table.

By repeated precipitation from acetone solutions with dry ether and subsequent crystallization from methanol, fraction 3 yielded thiobinupharidine perchlorate and a perchlorate with mp 225°–226° C. The new base from this perchlorate, which we have called nuphleine (II), with R_f 0.79, has the composition $C_{30}H_{42}O_4N_2S$ and mol. wt. 526 (mass-spectrometrically). The molecule of the alkaloid contains four oxygen atoms, two of which are present in two hydroxy groups, the presence of which was confirmed by the IR spectrum (3450 cm^{-1}) and by a determination of labile hydrogen; the other two oxygen atoms are present in furan rings, the presence of these being shown in the IR spectrum of the substance by absorption bands at 2128 cm^{-1}, 3165, 1600, 1579, 1508, 1379, 1240, 1025, 873, 852, 755 cm^{-1} and by the presence in the NMR spectrum of nuphleine in the region of aromatic protons of two signals with δ 7.18 (7–2 Hz) and 6.30 ppm due, respectively, to the protons in the α and β positions of a furan ring, the intensity of the signals from the α protons being twice as great as that from the β protons.

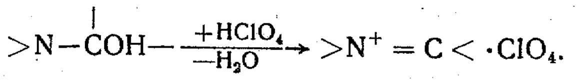
The composition of nuphleine and the presence in this molecule of furan rings permits the assumption that a genetic connection exists between nuphleine and deoxynupharidine and that nuphleine may be a bicyclic compound in which the linkage is effected through sulfur.

The catalytic hydrogenation of nuphleine with a platinum catalyst at 20° C or on heating to 100° C in ethanol or glacial acetic acid or on heating to 180° C with a hydrogen pressure of about 100 atm, and also with Raney catalyst in ethanolic solution, did not lead to any change in the alkaloid.

When nuphleine (II) was reduced with $NaBH_4$, two hydroxy groups were reduced to give a quantitative yield of thiobinupharidine (I), $C_{30}H_{42}O_2N_2S$. Such a conversion could take place if the hydroxyl in the nuphleine were on carbon atoms in the α position to nitrogen: $-\overset{|}{N}-\underset{|}{\overset{|}{C}}OH \rightarrow -\overset{|}{N}-\overset{|}{C}H-$.

Nuphleine perchlorate has the composition $C_{30}H_{38}O_2N_2S \cdot 2HClO_4$, and the IR spectrum of this substance has no absorption bands for hydroxy groups but has absorption bands characteristic for $>N^+=C<$ groups (1664, 1710 cm^{-1}).

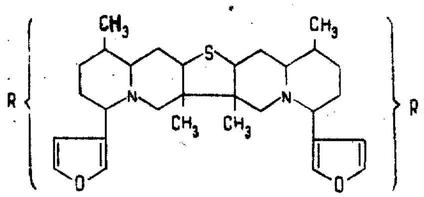
The difference in the elemental composition of nuphleine and its perchlorate (by H_4O_2), the absence of hydroxy groups in nuphleine perchlorate, and the presence in the IR spectrum of the perchlorate of bands of the $>N^+=C<$ groupings confirms the presence of an α-carbinolamine grouping in the molecule of nuphleine, for which the formation of anhydronium salts is characteristic:



$$>N-\overset{|}{C}OH \xrightarrow[-H_2O]{+HClO_4} >N^+=C< \cdot ClO_4.$$

This also confirms the fact that nuphleine differs from thiobinupharidine by the two hydroxy groups in the α-carbinolamine groupings.

For thiobinupharidine, Achmatowicz, et al., [7] have proposed a structural formula based on a study of the mass spectrum of the alkaloid and a comparison of this with the mass spectrum of deoxynupharidine taken as a model substance. These results, and also those which we have obtained, permit the structural formula (II) to be assumed for nuphleine. The position of the α-carbinolamine groups cannot be determined unambiguously.



I R = H
II R = OH

Experimental

Isolation of the alkaloids. Ten kilograms of the dry ground rhizomes of Nuphar luteum was steeped in 10% ammonia and the alkaloids were exhaustively extracted with dichloroethane. The alkaloids were extracted from the dichloroethane solution with dilute sulfuric acid by the generally accepted method. After the sulfuric acid solution had been made alkaline with ammonia, the alkaloids were extracted with chloroform, and the extract was evaporated to dryness. This gave 73.5 g of dry total bases.

Separation of the alkaloids. 73.5 g of the dry total alkaloids ground to a powder was chromatographed in a column filled with 2.2 kg of alumina (activity grade II, III). The alkaloids were eluted with ether and then with a mixture of ether and chloroform and with chloroform. Chromatographically homogeneous fractions were combined, the eluate was extracted with 3% hydrochloric acid, and a saturated aqueous solution of sodium perchlorate was added to the acid solution of the alkaloids. The precipitate of alkaloid perchlorates was separated off, carefully washed with water, and dried. Three perchlorate fractions were obtained.

Fraction 1 (eluted with ether). Chromatography of the eluate showed that the fraction is a mixture of thiobinupharidine (R_f 0.68) and neothiobinupharidine (R_f 0.59) with a clear predominance of the former alkaloid. The yield of the mixture of perchlorates was 3 g.

Fraction 2 (eluted with ether–chloroform). The eluate contained thiobinupharidine (R_f 0.68) and nuphleine (R_f 0.79) with the former predominating. Yield of the mixture of perchlorates was 32.4 g.

Fraction 3 (eluted with chloroform). The eluate contained thiobinupharidine (R_f 0.68) and nuphleine (R_f 0.79) with the latter predominating. Yield of the mixture of perchlorates was 45.7 g. The subsequent work was carried out with the fractions 1 and 3.

Thiobinupharidine. 3 g of fraction 1 was suspended in 10% ammonia and the bases were extracted with ether, giving, after evaporation, a mixture of alkaloids (1.95 g) which was triturated with 2 ml of 15% hydrochloric acid. A crystalline hydrochloride was obtained and from it 0.45 g of thiobinupharidine with mp 129°–130° C (from methanol), $[\alpha]_D$ +48.8° (c 1.1; ethanol), was obtained.

Found, %: C 72.96, 72.67; H 8.56, 8.64; N 5.62, 5.69; S 6.85, 7.07; mol. wt. 494 (mass spectrometrically). Calculated for $C_{30}H_{42}O_2N_2S$, %: C 72.85; H 8.56; N 5.65; S 6.50.

Thiobinupharidine perchlorate, obtained by mixing a solution of the alkaloid in 3% hydrochloric acid with a saturated solution of sodium perchlorate, melted at 282°–283° C (from methanol).

Neothiobinupharidine. The acid mother liquor after the extraction of the thiobinupharidine hydrochloride was extracted with chloroform, the evaporation of which gave impure neothiobinupharidine hydrochloride. The base, liberated from the hydrochloride (0.3 g) and recrystallized from absolute ethanol, melted at 158°–159° C.

Found, %: C 73.18, 73.38; H 8.57, 8.70; N 5.98, 5.81; S 6.39; mol. wt. 498 (cryoscopically). Calculated for $C_{30}H_{42}O_2N_2S$, %: C 72.85; H 8.56; N 5.65; S 6.50; mol. wt. 494.

Neothiobinupharidine perchlorate, obtained as described above, decomposed at 315°–320° C (from ethanol).

Nuphleine. A solution of 45.7 g of fraction 3 in 450 ml of acetone was treated with 15 ml of dry ether. After 2–3 days, a crystalline precipitate deposited which, according to a chromatogram, consisted of nuphleine. After recrystallization from methanol, 3.5 g of nuphleine perchlorate was obtained with mp 225°–226° C.

Found, %: C 52.49, 52.84; H 6.19, 6.05; N 3.82, 3.86; S 4.47, 4.62; Cl 10.20, 10.27. Calculated for $C_{30}H_{38}O_2N_2S \cdot 2HClO_4$, %: C 52.10; H 5.83; N 4.05; S 4.63; Cl 10.25.

The base isolated from the nuphleine perchlorate was amorphous.

Found, %: C 68.81, 68.78; H 8.29, 8.46; N 5.58, 5.56; S 6.08, 6.32; H_{labile} 0.37, 0.39: mol. wt. 526 (mass spectrometrically). Calculated for $C_{30}H_{42}O_4N_2S$, %: C 68.58; H 8.03; N 5.32; S 6.08; H_{labile} 0.19; mol. wt. 526.

Reduction of nuphleine with $NaBH_4$. A solution of 0.5 g of nuphleine in 15 ml of methanol was treated with 0.25 g of $NaBH_4$. After 20 hr, the methanol was evaporated off, an excess of water was added to the residue, and the reaction product was extracted with ether. This gave 0.48 g of a substance which, after crystallization from methanol, melted at 129°–130° C. By its constants, the absence of a depression of the melting point of a mixture with thiobinupharidine, and by the coincidence of their IR spectra, the substance was identical with thiobinupharidine.

The microanalytical and spectral investigations were carried out by E. A. Nikonova and M. E. Perel'son.

Summary

1. The dry rhizomes of *Nuphar luteum* L. have yielded three sulfur-containing alkaloids: two – thiobinupharidine and neothiobinupharidine – were known substances, the third with composition $C_{30}H_{42}O_4N_2S$ being new and having been given the name of nuphleine.

2. A possible structure has been proposed for nuphleine.

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