Phytochemistry, 1972, Vol. 11, pp. 3305 to 3306. Pergamon Press. Printed in England.

SULFUR CONTAINING ALKALOIDS FROM *NUPHAR LUTEUM**

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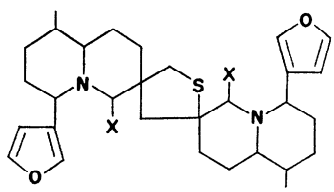
(*Received* 16 *May* 1972. *Accepted* 13 *June* 1972)

Key Word Index—*Nuphar luteum*; Nympheaceae; sulfur containing alkaloids; thionuphlutine-A; neothiobinupharidine; thiobinupharidine.

Abstract—Alkaloids neothiobinupharidine and thionuphlutine-A were isolated from rhizomes of *Nuphar luteum* obtained from Poland. These two alkaloids were not detected in the two North American *Nuphar* examined. However a bishemiaminal form of thionuphlutine-A has been isolated from North American *Nuphar*. Thionuphlutine-A is identical with the previously reported thiobinupharidine.

Nuphar luteum produces a number of sulfur-containing, C_{30} alkaloids. Among such alkaloids isolated from the rhizomes of *N. luteum* of East European origin[1] were neothiobinupharidine (I, X = H), the structure of which was studied by X-ray methods,[2] and thiobinupharidine, hitherto an alkaloid of unknown structure.[1]



(I)

We have examined *N. luteum* from Poland using a slightly modified version of the isolation procedure used by a group of Russian workers who reported the isolation of thiobinupharidine and neothiobinupharidine from *N. luteum* collected in the Ukraine and in Krasnodarsk Krai.[3] Our sample of neothiobinupharidine was identified by m.p., IR and NMR comparison with literature values.[1,3] The appearance of the CH_2S resonance at 2·70 δ and two separate resonance bands for the two β-furyl protons was of major assistance in identification.

The second sulfur-containing alkaloid which we isolated from the Polish *Nuphar* was thionuphlutine-A (I, X = H), a stereoisomer of neothiobinupharidine, and previously prepared through metal hydride reduction of 6,6′-dihydroxythionuphlutine-A (I, X = OH).[4]

* Support of this work by the National Institutes of Health, U.S. Public Health Service (Grant AI10188) is gratefully acknowledged.

[1] O. ACHMATOWICZ, H. BANASZEK, G. SPITELLER and J. T. WRÓBEL, *Tetrahedron Letters* 924 (1964); and references therein.
[2] G. I. BIRNBAUM, *Tetrahedron Letters* 4149 (1965).
[3] T. N. IL'INSKAYA, A. D. KAZOVKOV and T. G. MONAKHOVA, *Khim. Prir. Soedin.* **3**, 178 (1967).
[4] R. T. LALONDE, C. F. WONG and W. P. CULLEN, *Tetrahedron Letters* 4477 (1970).

Identification of thionuphlutine-A was made by IR, NMR, TLC, m.p. and m.m.p. comparison. 6,6′-Dihydroxythionuphlutine-A had been isolated from *N. luteum* subsps. *macrophyllum* and *variegatum*[5] obtained in New York. Interestingly, we have detected neither neothiobinupharidine nor thionuphlutine-A in the two North American *Nuphar*. Only the bishemiaminal 6,6′-dihydroxythionuphlutine-A was detected along with stereoisomeric 6,6′-dihydroxythionuphlutine-B, (I, X = OH).

Comparison of the various properties of thionuphlutine-A with those reported for thiobinupharidine strongly suggested that the two alkaloids were identical. Indeed, a direct comparison (NMR, IR, MS, m.p. and m.m.p.) of the two revealed that thionuphlutine-A and thiobinupharidine are in fact the same.[6,7] The name 'thionuphlutine-A' should therefore be deleted from the literature.

EXPERIMENTAL

Isolation of thionuphlutine-A (TN-A) and neothiobinupharidine (NTBN). Nuphar luteum rhizomes were collected near Galwiecie in the State of Bialostock, Poland and were obtained through the offices of Dr. L. Witkowska-Zük, Faculty of Forestry, The University of Agriculture, Warsaw, Poland. Powdered rhizomes, 950 g, were moistened with 1 l. of 10% aq. NH_3 and shaken with 4, 2 and 1 l. quantities of CH_2Cl_2 at 25°. The combined CH_2Cl_2 extract was treated with 2 and 1 l. of 10% aq. H_2SO_4. The combined aq. layer was brought to pH 14 with NaOH at 0°, saturated with Na_2SO_4 and extracted with CH_2Cl_2. The combined extract was dried and concentrated to 5·26 g of brown foam which was dissolved in 5 ml of CH_2Cl_2 and passed through 30 g of neutral Alumina (act. II). Evaporation of the CH_2Cl_2 eluant gave 3·5 g of brown oil which was rechromatographed on neutral alumina (act. III) with hexane, C_6H_6 (6× 100 ml fractions) and then solvents of increasing polarity. The first of the benzene fractions was rechromatographed twice on alumina (act. II, 5% Et_2O in hexane) to obtain 83 mg of material which was recrystallized from EtOH to give 58 mg of TN-A: m.p. 131–132·5°, m.m.p. TN-A[4] 131–132·5°; $[\alpha]_D^{25}$ +5·2°. TLC (alumina, 5% Et_2O in C_6H_6) R_f 0·7, (alumina, 10% Et_2O in hexane) R_f 0·55; IR, 3·63, 6·30, 6·66, 6·90, 7·00, 7·25, 7·30, 7·70, 7·79, 8·14, 8·68, 8·83, 8·92, 9·11, 9·44, 9·73, 9·82, 11·52, 13·89 μ; NMR ($CDCl_3$, 60 MHz) δ 0·94 (*d*, 6H, $HCC\underline{H}_3$), 2·33 (ABq, 2, H, $C\underline{H}_2S$), 2·75–3·1 (*m*, 4H, C-4H, C-4′, C-6 eq H and C-6′ eq H), 6·41 (*m*, 2H, β-furyl H) 7·35 (*m*, 4H, β-furyl H); MS *m/e* (% rel. int.) 494 (M^+) (39), 493 (6) 447 (5), 360 (5) 357 (4), 230 (53), 178 (100), 107 (17·8), 94 (14·5); perchlorate m.p. 175–180°; $[\alpha]_D^{25}$ +62° (95% EtOH, *c*, 208 m/g 10·0 ml). M.m.p., TN-A and thiobinupharidine[6] 131·5–132·5°.

Fractions 4–6 of the above described six, 100-ml C_6H_6 fractions were combined to obtain 98 mg of crystalline solid. This was chromatographed on 5 g of neutral alumina (act. II) with hexane, 10% Et_2O–hexane, C_6H_6 and 10% Et_2O–benzene. The crystalline solid (60 mg) eluted with C_6H_6 was neothiobinupharidine:[2] m.p. 158–160°; $[\alpha]_D^{25}$ −165° (95% EtOH, *c*, 0·4); TLC (alumina, CH_2Cl_2–Et_2O, 14:1) R_f 0·5 (alumina, hexane–Et_2O, 8:1) R_P 0·2; IR/CCl_4 3·64, 6·32, 6·69, 6·93, 6·99, 7·26, 7·31, 7·42, 7·59, 7·70, 7·79, 7·88, 8·60, 8·67, 9·02, 9·12, 9·42, 9·79, 10·39, 11·49, 13·87 μ; NMR ($CDCl_3$, 60 MHz) 0·91 (*m*, 6H, $HCC\underline{H}_3$), 2·70 (br *s*, 2H, $C\underline{H}_2S$) and superposed on 2·58–3·10 (*m*, 4H, NCH), 6·39 (*m*, 1H, β-furyl H), 6·58 (*m*, 1H, β-furyl H), 7·12–7·47 (*m*, 4H, α-furyl H).

Rhizomes of *N. luteum* subsp. *macrophyllum* were harvested from the lower Hudson River near Columbianville, New York during August 1968. Rhizomes of *N. luteum* subsp. *variegatum* were taken from the Southern shore of Green Lake, Onondaga County, near the village of Tully, New York during July and August 1967. Verification of both plant materials was made by Mr. S. Smith of the New York State Botanists Office, Albany, New York. The procedures employed in searching for the sulfur-containing alkaloids in the North American *Nuphar* were virtually the same as that described above for the Polish *Nuphar*.

[5] E. O. Beal, *J. Elisha Mitchell Sci. Soc.* **72**, 317 (1956).

[6] We are grateful to Dr. J. Wróbel, The University of Warsaw for providing us with a sample and NMR spectrum of thiobinupharidine.

[7] Since nuphleine is reduced[3] to thiobinupharidine (thionuphlutine-A) and the melting point of its diperchlorate agrees with that which we observed for 6,6′-dihydroxythionuphlutine-A,[4] it seems almost certain that nuphleine and 6,6′-dihydroxythionuphlutine-A are identical.