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THE STRUCTURES OF STAPHIDINE, STAPHININE, AND STAPHIMINE, THREE NOVEL BIS-DITERPENE ALKALOIDS FROM DELPHINIUM STAPHISAGRIA

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We wish to report here a successful application of ^{13}C and ^{1}H nmr spectroscopy to the structure determination of three new bis-diterpene alkaloids isolated from the mother liquors of Delphinium staphisagria. These new alkaloids are designated as staphidine (2), staphinine (3), and staphimine (4).

In 1941 Jacobs and Craig isolated a diterpene alkaloid named "staphisine" from the mother liquors accumulated during the isolation of delphinine from the seeds of D. staphisagria[1]. On the basis of chemical studies[1,2], they postulated that "staphisine" is a diterpene alkaloid dimer with molecular formula $C_{44}H_{60}N_2O$ (later revised to $C_{42}H_{60}N_2O$)[3], which contains no methoxyl group[1] (despite 1.36% OCH_3) and two N-CH_3 groups. During the chromatographic isolation of "staphisine", Jacobs and Craig found that the combustion analysis data of several samples of "staphisine" fluctuated between the limits of 82.13 and 82.85% for carbon and 9.47 and 9.77% for hydrogen. Attempts to separate "staphisine" by crystallization of the nitrate, hydrochloride, and hydrobromide salts were unsuccessful. They cautioned that "the so called staphisine could still be a persistent mixture of bases which are very difficult to separate". From these mother liquors, we recently isolated, by chromatography and crystallization, a methoxyl-containing bis-diterpene alkaloid, which we designated as staphisine[4], $C_{43}H_{60}N_2O_2$, and determined its structure as 1 by a single-crystal X-ray analysis of the monomethiodide[5]. We now find that Jacobs' "staphisine" is a mixture of 1 and a companion, non-methoxyl bearing alkaloid (2), named staphidine ($C_{42}H_{58}N_2O$). In addition, we have isolated two new imine-containing bis-diterpene alkaloids named staphinine ($C_{42}H_{56}N_2O_2$) and staphimine ($C_{41}H_{54}N_2O$).

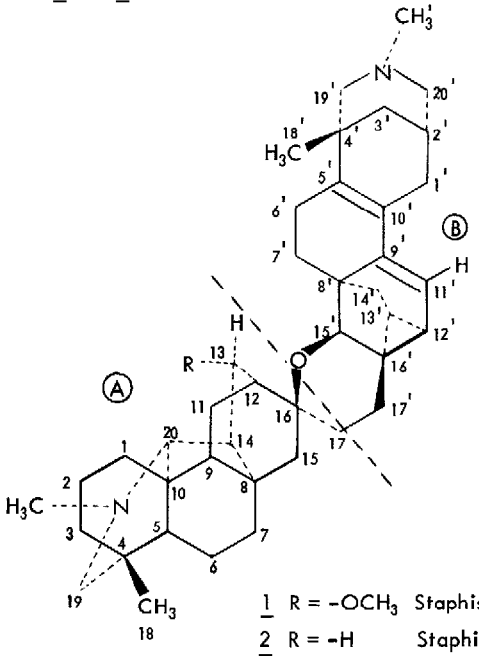
Staphidine, mp 213-216°, $[\alpha]^{24}D$ - 160° (c 2.0, benzene), shows absorption at λ_{max} (95% EtOH) 268 nm (ϵ 17,300). The ir spectrum shows no NH or OH absorption and weak absorption at 1710 and 1630 cm^{-1}. The mass spectrum exhibits an intense molecular ion peak at m/e 606 corresponding to the molecular formula $C_{42}H_{58}N_2O$[6]. Comparison of the mass spectrum of staphidine with that of staphisine (1) showed essential identity except for the appearance of a molecular ion peak at m/e 636 in staphisine.

The ^{1}H nmr spectrum of staphidine reveals the presence of two angular methyl groups (δ 0.91), two N-methyl groups (δ 2.13 and 2.21) and a vinyl proton (δ 5.85). A comparison of the ^{1}H nmr spectrum of staphidine with compounds 1, 3, and 4 appears in Table 1. In comparison with 1, staphidine shows the absence of a methoxyl singlet at δ 3.30 and an upfield shift of one N-methyl group from δ 2.27 to δ 2.21. This observed change ($\Delta\delta$ = 0.06 ppm) in the chemical shift of the N-methyl can be explained by the steric interaction between the N-CH_3 and -OCH_3 group in the A unit of staphisine. On the basis of the above

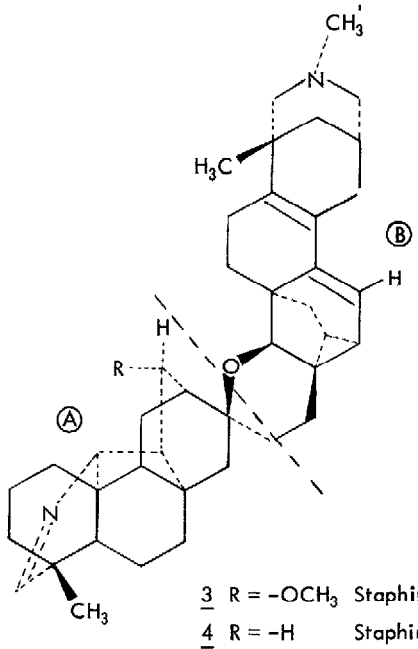
data, we can assign the chemical shift at δ 2.13 to the N-methyl group in unit B of the molecule, and at δ 2.27 and δ 2.21 to the N-methyl group in unit A in staphisine 1 and staphidine 2, respectively.

Further correlation of staphidine with 1 was made through a study of their ^{13}C nmr spectra (Table 2). Unambiguous carbon signal assignment in staphisine 1, was achieved by using conventional techniques, additivity relationships, and a direct analysis of nonprotonated carbon centers[7]. Comparison of ^{13}C nmr spectra of staphisine and staphidine afforded evidence for the absence of a methoxyl group at 57.8 ppm and methine carbon (C-13) at 89.4 ppm in compound 2. The C-13 chemical shifts of the remaining carbons of 1 and staphidine were in perfect agreement. Based on these data, structure 2 is assigned to staphidine. These results demonstrate that the alkaloid "staphisine", described by Jacobs and Craig[1] is in fact a mixture of the alkaloids we have designated as staphisine (1) and staphidine (2).

Staphinine, $[\alpha]^{24}D$ - 57.5° (c 1.0, benzene), which was not obtained in crystalline form[8] shows absorption at λ_{max} (95% EtOH) 268 nm (ε 17,300) in agreement with a transoid heteroannular conjugated diene system. The ir spectrum of staphinine shows absorption at 1720 (conj. diene), 1640 (-C=N-), 1101, and 1063 (ether linkage) cm^{-1}. The ^1H nmr spectrum indicates the presence of two angular methyl groups (δ 0.94 and 1.00), only one N-methyl group (δ 2.13), a methoxyl group (δ 3.30), a vinyl proton (δ 5.85) and an imine proton (δ 7.30). Staphimine, $[\alpha]^{24}D$ - 58.5° (c 1.0, benzene), which also was not obtained in crystalline form[8], showed ir and uv spectra which are very similar to those of staphinine. The ^1H nmr spectrum was also identical in all respects except for the absence of a methoxyl singlet at δ 3.30 (Table 1). The above data indicate that staphinine and staphimine are very similar to each other and are related to alkaloids 1 and 2, respectively.



1 R = -OCH_3 Staphisine
2 R = -H Staphidine



3 R = -OCH_3 Staphinine
4 R = -H Staphimine

Table 1. ^1H NMR Chemical Shifts of Staphisine 1, Staphidine 2, Staphinine 3 and Staphimine 4[a]

Carbon	1	2	3	4
$-C-CH_3^{18}$	0.91	0.91	1.00	1.00
$-C-CH_3^{18'}$	0.91	0.91	0.94	0.94
$N-CH_3'$	2.13	2.13	2.13	2.13
$N-CH_3$	2.27	2.21	-	-
$-OCH_3$	3.30	-	3.30	-
-C=CH-	5.85	5.85	5.85	5.85
-N=CH-	-	-	7.30	7.30

a) ^1H NMR spectra were determined in $CDCl_3$ and shifts are given on the δ scale relative to TMS.

Table 2. Carbon-13 Chemical Shifts of Staphisine 1, Staphidine 2, Staphinine 3 and Staphimine 4[a]

Carbon	1	2	3	4
C-4	34.2(s)	34.2(s)	41.5(s)	41.5(s)
C-8	37.4(s)	37.6(s)	38.1(s)	38.3(s)
C-10	46.0(s)	45.5(s)	44.3(s)	43.7(s)
C-13	89.4(d)	-	91.2(d)	-
C-16	72.2(s)	73.6(s)	72.3(s)	73.8(s)
C-19	60.7(t)	60.4(t)	168.1(d)	167.6(d)
C-20	74.4(d)	77.0(d)	73.1(d)	75.8(d)
$N-CH_3$	43.9(q)	43.5(q)	-	-
$C-OCH_3$	57.8(q)	-	56.4(q)	-
C-4'	34.5(s)	34.4(s)	34.4(s)	34.5(s)
C-5'[b]	135.6(s)	135.6(s)	135.5(s)	135.7(s)
C-8'	41.8(s)	41.6(s)	41.6(s)	41.6(s)
C-9'[b]	127.6(s)	127.7(s)	127.7(s)	127.9(s)
C-10'[b]	135.6(s)	135.8(s)	135.5(s)	135.7(s)
C-11'	112.9(d)	112.7(d)	112.9(d)	113.3(d)
C-15'	78.1(d)	77.6(d)	78.5(d)	77.9(d)
C-16'	29.5(s)	29.3(s)	29.5(s)	29.4(s)
C-19'[c]	62.5(t)	62.4(t)	62.5(t)	62.3(t)
C-20'[c]	64.7(t)	64.5(t)	64.7(t)	64.4(t)
$N-CH_3'$	46.6(q)	46.3(q)	46.3(q)	46.4(q)

[a] Carbon-13 spectra were taken at 25.03 MHz in the Fourier mode using a JEOL-PFT-100 spectrometer in conjuction with a EC-100-20K memory computer. Samples were dissolved in $CDCl_3$ containing TMS as an internal standard. Concentrations were about 0.3-0.8M. [b,c] These assignments may be reversed.

The carbon-13 chemical shifts for staphinine, staphimine, and alkaloids 1 and 2 appear in Table 2. The pattern of carbon-13 chemical shifts in these alkaloids is very similar to that of 1 and 2 except for a few changes. The presence of an imine (-CH=N-) group in staphinine and staphimine was established by comparison with ^{13}C nmr chemical shifts of known atisine derivatives containing an imine group (e.g. atisine azomethine shows 166.4 ppm). The lack of one N-methyl group and the absence of a chemical shift in the region of 60 ppm (C-19) in these alkaloids (compared with 60.7 and 60.4 ppm for 1 and 2, respectively) is explained by the presence of an imine group (167.6 ppm, C-19). The downfield shift (7.3 ppm) of the C-4 carbon and the upfield shifts (1.3 and 1.2 ppm) of the C-20 carbon in 3 and 4 relative to 1 and 2, respectively, are due to the presence of an imine group in the A unit. This was also confirmed on the basis of an N-methyl singlet at δ 2.13 in the ^{1}H nmr spectrum of both 3 and 4. Based on the arguments presented here, structures 3 and 4 are proposed for staphinine and staphimine, respectively.

Staphinine and staphimine occur in the seeds of D. staphisagria to the extent of about 5% of the combined weight of staphisine and staphidine. It is appealing to consider that staphinine (3) and staphimine (4) may be biogenetic precursors of staphisine (1) and staphidine (2), respectively. Because the nitrogen bridge connects atoms 4 and 10 in the A unit, and atoms 4' and 2' in the B unit, it is unlikely that these alkaloids are synthesized in the plant by dimerization of two identical atisine-type units[9].

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References and Notes

1. W. A. Jacobs and L. C. Craig, J. Biol. Chem., 141, 67 (1941).
2. L. C. Craig and W. A. Jacobs, ibid, 152, 645 (1944).
3. C. H. Huebner and W. A. Jacobs, ibid, 169, 211 (1947).
4. Subsequent examination of the sample of staphisine, mp 200-208°, reported earlier[5], shows that it is still a mixture of 1 and 2. However, the monomethiodide crystal selected for a single crystal X-ray analysis consisted mainly of the monomethiodide of compound 1. In the future we will utilize the name staphisine only to designate the alkaloid of structure 1, mp 211-213°, and staphidine to indicate the desmethoxy alkaloid 2, mp 213-216°.
5. S. W. Pelletier, A. H. Kapadi, L. H. Wright, S. W. Page and M. Gary Newton, J. Amer. Chem. Soc., 94, 1754 (1972).
6. Elemental analyses for C, H and N showed satisfactory agreement with the stated empirical formulas. The melting points are corrected and were taken on a hot-stage microscope equipped with a polarizer.
7. E. Wenkert, A. O. Clouse, D. W. Cochran, and D. Doddrell, J. Amer. Chem. Soc., 91, 6879 (1969)
8. Staphinine 3 and staphimine 4 are amorphous and extremely sensitive to heat and light in comparison with staphisine 1 and staphidine 2.
9. We were unable to carry out any transformation of compounds 3 and 4 to 1 and 2, respectively, due to the unstability of 3 and 4 toward various reagents (e.g., $NaBH_4$, $NaBH_3CN$, etc.).