Tetrahedron Letters No. 21, pp 1749 - 1752, 1976. Pergamon Press. Printed in Great Britain.

THE STRUCTURES OF STAPHISAGNINE AND STAPHISAGRINE, TWO NOVEL BIS-DITERPENE ALKALOIDS FROM DELPHINIUM STAPHISAGRIA

S. William Pelletier*, Zoltan Djarmati, and Naresh V. Mody

Natural Products Laboratory, Department of Chemistry, University of Georgia, Athens, Georgia 30602

(Received in USA 9 March 1976; received in UK for publication 16 April 1976)

We wish to report the structures of staphisagnine (1) and staphisagrine (2), two new bis-diterpene alkaloids isolated from the mother liquors of Delphinium staphisagria. These compounds are unusual in containing an oxazolidine ring in addition to many of the uncommon features of the staphisine (3) skeleton.

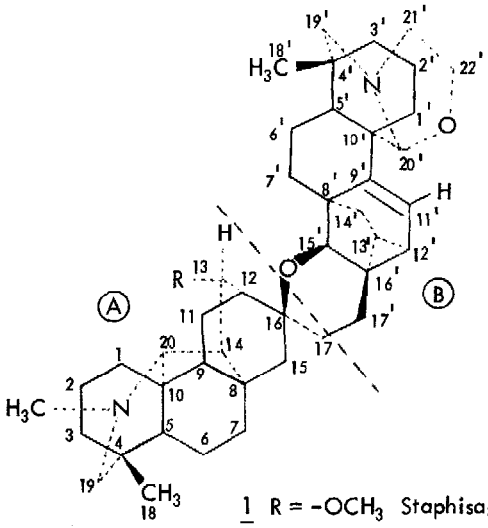
The mother liquors which had been accumulated during the isolation of delphinine from the seeds of D. staphisagria were found to contain a relatively large amorphous fraction of alkaloids[1]. We recently described[2] the isolation and structures of three new bis-diterpene alkaloids (staphidine, staphinine, and staphimine) from these mother liquors. We report now the isolation of two bis-diterpene alkaloids named staphisagnine (1) and staphisagrine (2), which contain an oxazolidine ring of the atisine and veatchine type.

Staphisagnine, $C_{44}H_{62}N_2O_3$, $[\alpha]^{25}$ D - 104.5° (c 2.0, benzene), was isolated as a resin. Its ir spectrum shows absorption at 1675 (-C=C-), 1105 and 1075 (ether linkage) cm^{-1}. The ^1H nmr spectrum in $CDCl_3$ reveals the presence of two angular methyl groups (δ 0.82 and 0.93), an N-methyl group (δ 2.27), a methoxyl group (δ 3.30), N-CH-O proton as part of an oxazolidine ring (δ 4.06), and a vinyl proton (δ 5.93). Staphisagrine, $C_{43}H_{60}N_2O_2$, mp 229-231°, $[\alpha]^{25}$ D - 105.6° (c 1.4, benzene) shows absorption at 1675 (-C=C-), 1105 and 1075 (ether linkage) cm^{-1} in its ir spectrum. The ^1H nmr spectrum in $CDCl_3$ shows the presence of two angular methyl groups (δ 0.82 and 0.93), an N-methyl group (δ 2.21), N-CH-O proton (δ 4.06) and a vinyl proton (δ 5.93). The ^1H nmr spectrum was identical with that of alkaloid 1 except for the absence of a methoxyl singlet at δ 3.30. These data indicate that staphisagnine and staphisagrine are closely related.

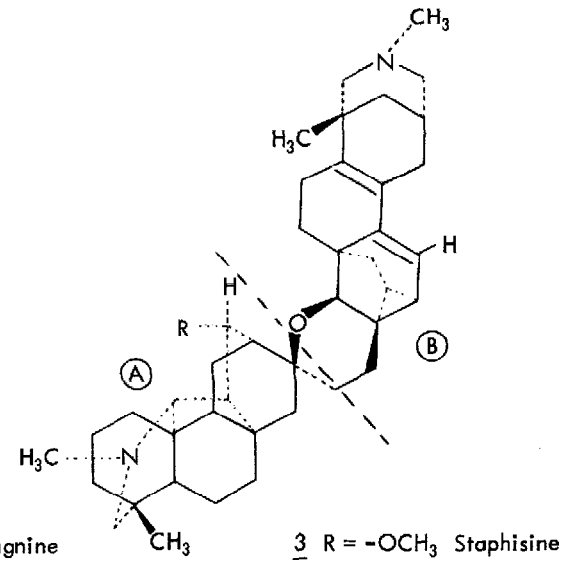
The ir, ^1H and ^{13}C nmr spectra of these new alkaloids show some similarity with the alkaloids staphisine 3 and staphidine 4[2,4]. Alkaloids 1 and 2 show one N-methyl group in the ^1H nmr spectra in contrast to two N-methyl groups in compounds 3 and 4. The absorptions at δ 2.27 in 3 and δ 2.21 in 4 have been assigned[2] to the N-methyl group in the A unit of the molecule. The N-methyl groups in 1 and 2 absorb at exactly the same position: δ 2.27 and δ 2.21, respectively. This result confirms the presence of an N-methyl group in the A unit of staphisagnine and staphisagrine. Both the absence of an N-methyl singlet at δ 2.13 and the presence of an oxazolidine ring in 1 and 2 indicate that substitution has taken place on the nitrogen in the B unit of these compounds. This result fixes the position of the oxazolidine ring in the B unit of alkaloids 1 and 2.

Staphisagnine 1 and staphisagrine 2 were further related to the known alkaloids (3 to 8) through a study of their ^{13}C nmr spectra. The ^{13}C chemical shift correlation diagram (Figure 1) shows the assignments in the staphisine-type bis-diterpene alkaloids 1 to 4. Assignment of the resonances to individual carbon atoms was achieved by using conventional techniques, chemical-shift theory, direct analysis of non-protonated carbon centers[5] and comparison with known alkaloids (5 to 8).

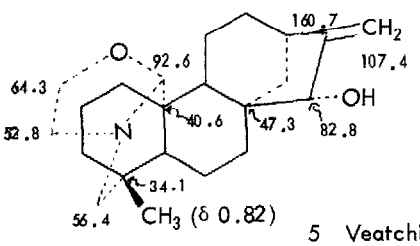
The lowfield resonances at 135.7, 135.5, 127.6 and 112.7 ppm in 3 and 4 are assigned to the conjugated double bonds which contain three quaternary and one methine carbons and are in agreement with results obtained by single crystal X-ray analysis of staphisine 3[4]. However, alkaloids 1 and 2 at lowfield show only singlets at 140.2 and 140.3 ppm and doublets at 117.9 and 117.8 ppm, respectively. This result



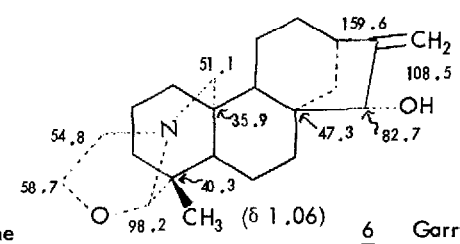
1 R = -OCH$_3$ Staphisagnine
2 R = -H Staphisagrine



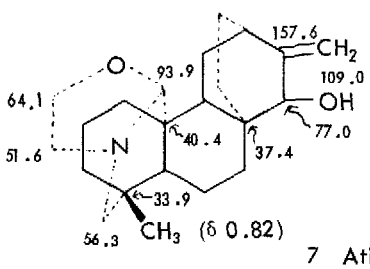
3 R = -OCH$_3$ Staphisine
4 R = -H Staphidine



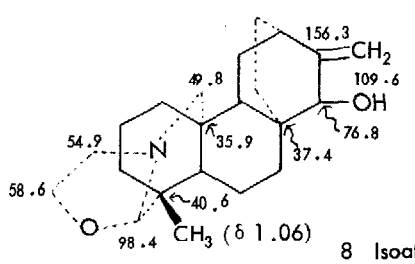
5 Veatchine



6 Garryine



7 Atisine



8 Isoatisine

indicates the presence of one similar double bond in staphisagnine and staphisagrine. The position of this double bond can be assigned to C-9' —— C-11' on the basis of the multiplet pattern of the vinyl proton signal in the ^{1}H nmr (similar pattern in 3 and 4) and the constant ^{13}C chemical shifts which are exhibited by C-8' (42.3 ± 0.7 ppm), C-15' (77.5 ± 0.6 ppm), and C-16' (29.2 ± 0.3 ppm) in alkaloids 1 to 4.

In the region between 70 to 90 ppm in the ^{13}C spectra of 1 and 3, there are four peaks in contrast to three peaks in 2 and 4. The extra resonance at 89.7 ppm in staphisagnine and at 89.4 ppm in staphisine 3 is assigned to C-13 to accommodate the presence of a methoxyl group. The remaining three resonances in the region between 70 to 90 ppm are assigned to C-15', C-16, and C-20 (Figure 1) of alkaloids 1 to 4. The signals in the region between 50 to 65 ppm play an important role in the structure determination of staphisagnine and staphisagrine. There are three N-methylene signals at 60.4, 62.4, and 64.5 ppm in 3 and similar signals in 4 (Figure 1). Two of these signals at 62.4 and 64.5 ppm were assigned to C-19' and C-20' of the B unit and the remaining signal at 60.4 ppm to C-19 of staphisine 3 and staphidine 4[2]. However, similar signals at 60.8 ppm in staphisagnine and 60.6 ppm in staphisagrine indicate that the A unit in these alkaloids is similar to that of known alkaloids 3 and 4.

Resonances for three hetero-substituted methylene carbons at 54.4, 55.3, and 58.0 ppm and the methine carbon at 100.0 ppm in staphisagnine and similar resonances in staphisagrine are due to the rearranged nitrogen center and the presence of an oxazolidine ring in unit B of the molecule. The latter was confirmed by the comparison of ^{13}C chemical shifts of known alkaloids veatchine 5[6], garryine 6[7], atisine 7[8-10], and isoatisine 8.

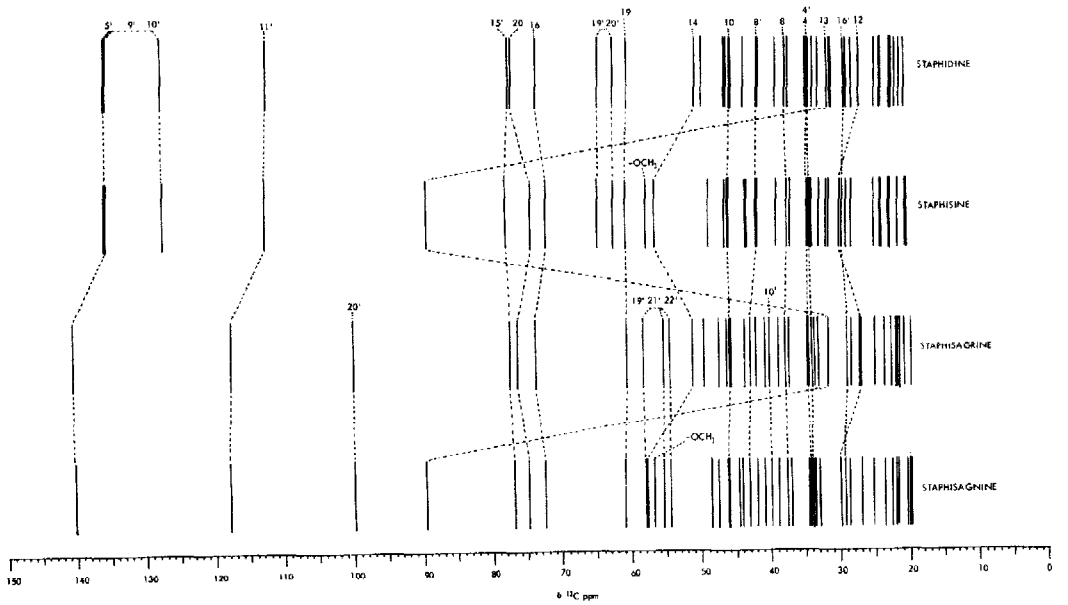


Figure 1. Correlation of carbon-13 chemical shifts for staphisagnine (1), staphisagrine (2), staphisine (3) and staphidine (4). Carbon-13 spectra were taken at 25.03 MHz in the Fourier mode using a JEOL PFT-100 spectrometer in conjuction with a EC-100-20K memory computer. Samples were dissolved in $CDCl_3$ containing TMS as an internal standard.

The presence of an extra singlet at 40.0 ppm (C-10') in staphisagnine and staphisagrine also indicates that a structural difference exists at this point in the molecule as compared with 3 and 4. In order to determine the presence of an iso- or a normal-type oxazolidine ring in these new alkaloids, further comparison of the ^1H chemical shift of the angular methyl group at C-4' was made with known alkaloids 5 to 8[11]. The methyl singlet (δ 0.82) assigned to C-18' in staphisagnine and staphisagrine is in perfect agreement with the methyl singlet (δ 0.82) assigned to the corresponding position (C-18) in veatchine 5 and atisine 7. Also the resonance of the C-4' carbon (34.1 ppm) in staphisagnine and staphisagrine is at the same position as found in veatchine and atisine (34.1 and 33.9 ppm, respectively). The unusual upfield ^{13}C chemical shift of C-20 at 92.6 ppm in veatchine 5 and at 93.9 ppm in atisine 7[12] in comparison with staphisagnine and staphisagrine (C-20' at 100 ppm) can be explained by the steric effect of the C-8 — C-14 bond[13]. These data indicate that staphisagnine and staphisagrine contain a normal type oxazolidine ring as present in veatchine 5 and atisine 7. From these results, we assign structures 1 and 2 to staphisagnine and staphisagrine, respectively.

Acknowledgment. We are grateful to Mr. Courtney Pape for providing the ^{13}C nmr spectra. We acknowledge a N.S.F. matching grant to the department for purchase of the ^{13}C-nmr spectrometer.

References and Notes

1. W. A. Jacobs and L. C. Craig, J. Biol. Chem., 141, 67 (1941).
2. S. W. Pelletier, N. V. Mody, Z. Djarmati, I. V. Mićović, and J. K. Thakkar, Tetrahedron Lett., in press (1976).
3. Elemental analyses for C, H, and N showed satisfactory agreement with the stated formulas. The melting point is corrected and was taken on a hot-stage microscope equipped with a polarizer.
4. S. W. Pelletier, A. H. Kapadi, L. H. Wright, S. W. Page, and M. Gary Newton, J. Amer. Chem. Soc., 94, 1754 (1972).
5. E. Wenkert, A. O. Clouse, D. W. Cochran, and D. Doddrell, J. Amer. Chem. Soc., 91, 6879 (1969).
6. K. Wiesner, R. Armstrong, M. F. Bartlett, and J. A. Edwards, J. Amer. Chem. Soc., 76, 6068 (1954); K. Wiesner, and J. A. Edwards, Experientia, XI, 255 (1955).
7. The C-19 and C-20 resonances in garryine 6 and isoatisine 8 are assigned on the basis of a deuterium exchange study.
8. K. Wiesner, R. Armstrong, M. F. Bartlett, and J. A. Edwards, Chemistry and Industry, 132 (1954).
9. S. W. Pelletier and W. A. Jacobs, J. Amer. Chem. Soc., 76, 4496 (1954); S. W. Pelletier and P. C. Parthasarathy, ibid., 87, 777 (1965).
10. J. W. ApSimon and O. E. Edwards, Canad. J. Chem., 40, 896 (1962); D. Dvornik and O. E. Edwards, ibid., 42, 137 (1964).
11. S. W. Pelletier and T. N. Oeltmann, Tetrahedron, 24, 2019 (1968).
12. Atisine exists in two different conformers in 2:1 ratio in $CDCl_3$ solution at room temperature[11]. The carbon-13 chemical shifts of the more stable conformer are given here.
13. S. W. Pelletier, K. Kawazu, and K. W. Gopinath, J. Amer. Chem. Soc., 87, 5229 (1965).