Tetrahedron Letters,Vol.26,No.23,pp 2759-2762,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

ISOLATION AND STRUCTURE DETERMINATION OF NIGELLICINE, A NOVEL ALKALOID FROM THE SEEDS OF NIGELLA SATIVA

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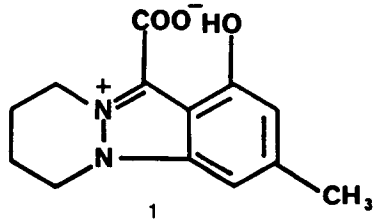
Summary: The structure of nigellicine (1), an unusual alkaloid from the seeds of Nigella sativa, was determined by x-ray diffraction and spectroscopic techniques.

Plants of the Indian subcontinent are a continuing source of fascinating natural products. Nigella sativa Linn. (Rannunculaceae), known in Pakistan as kalonji, is a widely distributed herbaceous plant. It generally grows in Mediterranean countries and is cultivated in Pakistan. While it is widely used as a spice and for the treatment of various diseases,[1-4] little work has been carried out on its alkaloidal constituents.[5-7] In this note we wish to report the isolation and structure determination of the unusual alkaloid shown as **1** to which we have given the trivial name nigellicine.



1

Nigella sativa seeds (20 kg) were soaked in ethanol (35 liters) and then crushed. This mixture was filtered and the solvent evaporated. The concentrate thus obtained was defatted with Et_2O, acidified with 5% HCl, filtered, made basic (pH 8-9) with concentrated NH_3, and extracted

with $CHCl_3$ (3x250ml). The $CHCl_3$ extract was evaporated to afford a brownish material which was chromatographed on an open column packed with silica gel-60. Elution with $CHCl_3$ followed by increasingly polar $CHCl_3$:MeOH mixtures afforded a number of fractions. The fraction from 96% $CHCl_3$: 4% MeOH afforded an amorphous material which was crystallized by addition of a small amount of ether. The yellow-colored crystals thus obtained were purified by preparative tlc on silica gel (GF-254) using $CHCl_3$:MeOH (9.4:0.6) as the solvent system. The material in the colored band was separated and crystallized from ethanol to afford optically inactive, yellow crystals. These crystals do not give a meaningful m.p. but decompose over a wide temperature range. The HRMS was initially interpreted as showing a molecular ion at m/z 202.1102 (calc. for $C_{12}H_{14}N_2O$, 202.1106). Subsequent spectra recorded using a field desorption technique showed this as a base peak and also revealed the true molecular ion peak at m/z 246 ($C_{13}H_{14}N_2O_3$). Prominent peaks in the MS appeared at m/z 246 (M^+, 20%), 202 (M^+-CO_2, 100%), 174 ($C_{10}H_{10}N_2O$, 13%), 173 ($C_{11}H_{13}N_2$, 14%), 160 ($C_9H_8N_2O$, 19%), 148 ($C_8H_8N_2O$, 18%), 131 ($C_8H_8N_2$, 25%), 119 ($C_7H_7N_2$, 16%), 104 (C_7H_6N, 12%), and 91 (C_7H_7, 13%). The UV spectrum (EtOH) showed absorptions at λ_{max}(nm) (log ε) 240 (4.30), 288 (3.52), 296 (3.51), 353 (3.53). There were minima at λ_{min}(nm) (log ε) 268 (3.31), 293 (3.51), and 312 (3.16). The IR (KBr) had absorptions at 3405 cm^{-1} (OH); 3055, 2910, 2890 (ArC-H, N-CH, C-H); 1670, 1650 (-CH=N, C=O); 1637, 1625 (C=C, Ar-C-C); 1576, 1406 ($-COO^-$); and 1286, 1266 (C-O, C-N). These and other spectral data (see below) indicated that nigellicine was a highly conjugated molecule of an unfamiliar structural type. We elected to unequivocally establish its structure by the single crystal x-ray diffraction technique.

Nigellicine could be recrystallized from aqueous methanol or ethanol in a form suitable for x-ray diffraction analysis. Preliminary x-ray photographs displayed monoclinic symmetry; and accurate lattice constants of **a**=7.1913(31), **b**=20.2380(44), **c**=18.4783(38) Å, and β=86.991(27)° were determined from a least-squares fit of fifteen diffractometer measured 2θ-values. Systematic extinctions and an approximate density indicated space groups C2 or C2/c with four molecules of composition $C_{13}H_{14}N_2O_3 \cdot 2H_2O$ in the unit cell. Successful refinement indicated that the correct choice was C2/c with one molecule in the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer controlled four-circle diffractometer using variable speed, 1° ω-scans and graphite monochromated Cu K$\bar{\alpha}$ radiation (1.54178 Å). A total of 1811 reflections were collected in this fashion; and, after correction for Lorentz, polarization and background effects, 883 (49%) were judged observed ($|F_o| \geq 3\sigma(F_o)$). A phasing model was easily found using direct methods.[8] Block-diagonal least-squares refinements with anisotropic nonhydrogen and isotropic hydrogen atoms have

converged to a conventional crystallographic residual of 0.064 for the observed data.[9]

Figure 1 is a computer generated perspective drawing of the final x-ray model without hydrogens. A conventional chemical drawing is given in **1**. Formulation **1** represents only one of the possible resonance and tautomeric structures of nigellicine, but it is the one most consistent with the observed bond distances and angles. For example, the carboxylate bond distances are essentially equal with C14-O15 equal to 1.233(9) Å and C14-O16, 1.256 Å. There is an intramolecular H-bond from O16 to HO17. The bond distances in the benzopyrazole fragment are characteristic of a delocalized aromatic system. Atoms N1 through C10 and C13 are planar, and C12 and C11 are above and below this molecular plane.

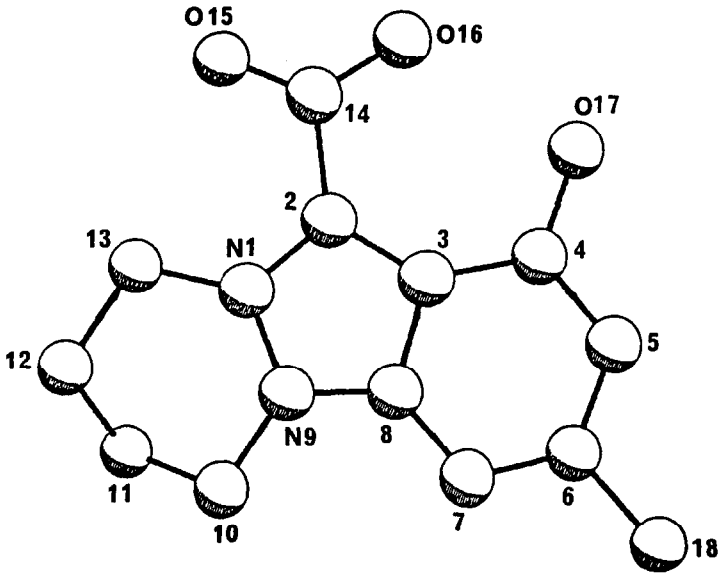


Figure 1. A computer generated perspective drawing of nigellicine (**1**). Hydrogens are omitted for clarity.

The structure shown in **1** is also consistent with the ^{1}H-NMR spectrum of nigellicine. Spectra recorded at 400 MHz ($CD_3OD/CDCl_3$) showed a four proton multiplet centered at δ 2.32 which was assigned to the methylenes at C11 and C12. A triplet at δ 4.46 was assigned to the methylene protons on C10. The methylene protons attached to C13 appeared as a low field triplet at δ 5.18 due to the adjacent quaternary nitrogen atom. The two aromatic protons were singlets at δ 6.75 (C7) and 6.58 (C5). The methyl singlet was at δ 2.48. The 2D-^{1}H-NMR (400 MHz, $CD_3OD/CDCl_3$) clearly indicated that the methylene protons of C10 and C13 (triplets at δ 4.46 and 5.18) were coupled to the methylene protons of C11 and C12 (multiplet at δ 2.32). The ^{13}C NMR (100.8 MHz, $CD_3OD/CDCl_3$) showed resonances at δ (tentative assignment) 141.9 (C3); 153.7 (C4); 97.6 (C5); 138.3 (C6); 110.5 (C7);

148.2 (C8); 46.8 (C10); 19.2 (C11); 20.0 (C12); 49.48 (C13); and 22.4 (C18). There was a remaining signal at 159.65 which is attributed to either C2 or C14.

We are aware of no natural products similar to nigellicine (**1**), and its biogenesis is obscure. The number of naturally occurring nitrogen-nitrogen bonds is small. In addition to nigellicine, two other alkaloids have been isolated from N. sativa in very small quantities. The tentative formulas of these, as determined by HRMS are $C_{12}H_{13}NO_2$ and $C_{19}H_{20}N_2O_2$. Further structural and synthetic studies are in progress.

ACKNOWLEDGMENTS. The Cornell workers wish to thank NSF INT 83-14133 and NIH CA24487 for financial assistance.

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4. This plant has an extensive historical background, and, according to Birdwood, it is the black cumin mentioned in the Bible, the melanthion of Hippocrates and Dioscorides, and the gith of Pliny. The Prophet Mohammad also mentioned its importance and called it a potent remedy for diseases.

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8. All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 78, MULTAN 80, and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from x-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq and M. M. Woolfson, University of York, England, 1978 and 1980; BLS78A, an anisotropic block diagonal least squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a crystallographic illustration program by W.D.S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.

9. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, ENGLAND CB2 1EW and are available from them. Include a complete literature citation when ordering.

(Received in USA 6 February 1985)