

Structure of Koumine

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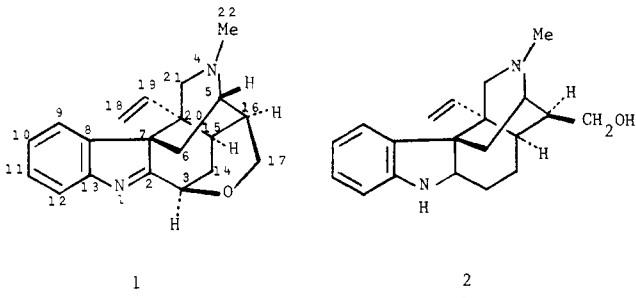
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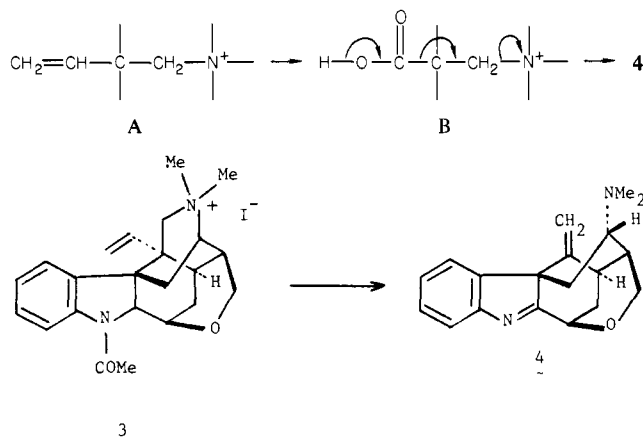
Koumine, $C_{20}H_{22}N_2O$, the principal alkaloid of the Chinese toxic medicinal plant Kou-wen (*Gelsemium elegans* Benth.),¹ was previously shown to contain an indolenine residue.² We now describe the complete structure and relative stereochemistry of this alkaloid (**1**).

The presence of a 2,7,7-trisubstituted indolenine nucleus in koumine was confirmed by its ¹H NMR spectrum, which shows four contiguous benzenoid protons at δ (CDCl₃) 7.61 (dd, H9), 7.36 (td, H10), 7.25 (td, H11), and 7.55 (dd, H12), with $J_{ortho} = 7.4$ Hz and $J_{meta} = 1.4$ Hz, and ¹³C NMR spectrum, which displays signals corresponding to the eight carbons of the indolenine at δ (CDCl₃) 185.7 (s, C2), 58.0 (s, C7), 143.7 (s, C8), 123.1 (d, C9), 126.0 (d, C10), 128.2 (d, C11), 121.2 (d, C12), 154.9 (s, C13). Support for these assignments was afforded by proton and off-resonance decoupling of ¹H and ¹³C signals due to C9-12 and by the observation that, in dihydrokoumine (containing a dihydroindole nucleus), the absorptions due to C2 and C7 are shifted upfield to δ 75.5 and 41.8, respectively.

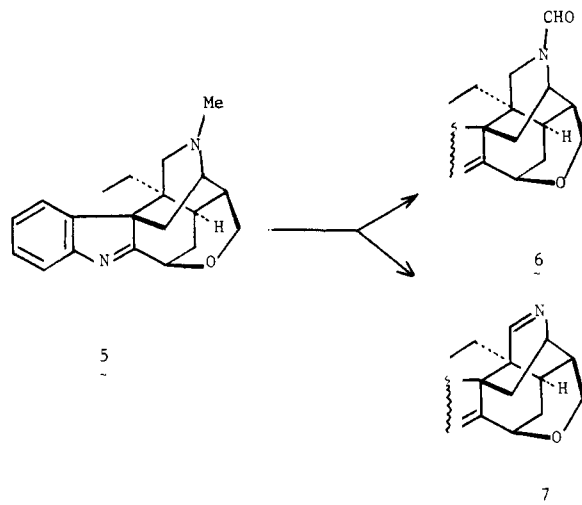
The ¹H NMR spectrum of koumine also shows a signal at δ 5.02 (H3, ddd, $J = 1.0, 2.4,$ and 3.5 Hz) which is shifted upfield to δ 4.22 (br d) in the dihydro derivative and an ABX system (H17, -17', -16) with δ 3.62, 4.26 ($J_{AB} = 12.2$ Hz), and 2.79 (m). Treatment of koumine with sodium in ethanol gave a primary alcohol, dihydrokouminol (**2**), which must arise by reductive cleavage of an ether. When taken with the NMR data, it implies that the indolenine nucleus is linked to the single oxygen atom of the alkaloid by a methine unit, and that koumine therefore contains the moiety $N=CC(O)HCH_2CH$.



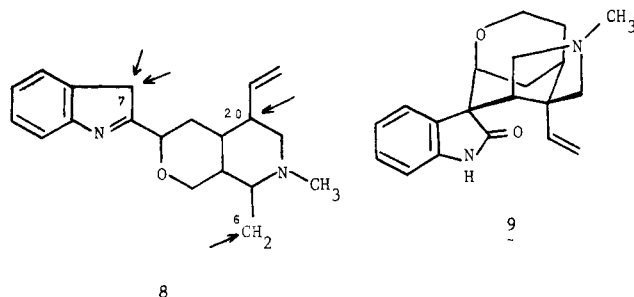
Oxidation of *N*-acetyldihydrokoumine methiodide (**3**) with aqueous potassium permanganate was accompanied by decarboxylation and furnished a product with the formula $C_{19}H_{22}N_2O$. According to its UV spectrum, which exhibited λ_{max} (EtOH) at 220 and 260 nm, this material contained the indolenine chromophore of koumine. More significantly, the presence of an exomethylene group in this product could be recognized from its ¹H NMR spectrum, which showed a pair of olefinic protons centered at δ 4.59, and IR spectrum which contained characteristic bands at 3070, 1643, and 895 cm^{-1} . In addition, the presence of an NMe_2 group was revealed by a six-proton signal at δ 2.33 (s, 6 H). Since it was known that koumine possesses a vinyl group, part structure A becomes tenable, so that oxidation to B, followed by an eliminative decarboxylation, gives **4**.³



The nature of the remaining substitution at the nonindolenine nitrogen of koumine was revealed by hydrogenation of the vinyl group to yield isodihydrokoumine (**5**), followed by oxidation with potassium permanganate in acetone.⁴ Two products were isolated, one neutral and the other basic. The neutral material **6** ($C_{20}H_{22}N_2O_2$) contained a formamide according to its IR (1660 cm^{-1}), ¹H NMR (δ 8.30), and ¹³C NMR (δ 160.7) spectra. In addition, a three-proton system (H6, -6', -5) with δ 2.26, 2.78 (each a dd), δ 3.87 (dt, 1 H) and $J = 14.2, 3.7,$ and 2.0 Hz was observed, which is consistent with a partial formulation $OHCNCHCH_2$. The basic product **7**, displayed H6, -6', -5 in its ¹H NMR spectrum at δ 1.70, 2.68 each a dd, and 4.53 (m) with $J = 13.6, 1.9,$ and 2.9 Hz, together with features characteristic of an imine [ν_{max} 1605 cm^{-1} and δ 8.41 (s, 1H)]. This implies that **7** incorporates a fragment $CH=NCHCH_2$ and thus corroborates the assignment to **6**. The connectivity of C5, C14, C15, C16, and C20 was established by double irradiation experiments on **1**, **4**, **6**, **7**, and the dihydro



derivative of **4**, with the result that the partial structure **8** (where arrows indicate residual bonds) was deduced for koumine. Recognition that koumine, containing five double bonds, must be hexacyclic then led to formula **1**.



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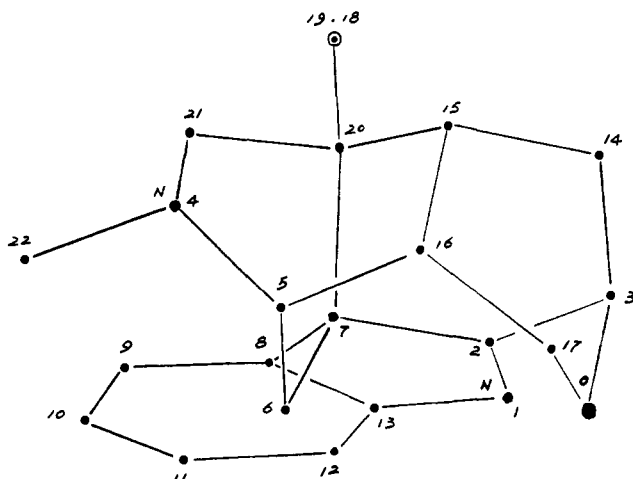


Figure 1. Three-dimensional projection of koumine hydrobromide from X-ray crystallographic analysis.

The relative configuration of koumine was also deduced from an analysis of the ^1H NMR spectrum of the alkaloid and its derivatives. Thus, the coupling constant between H3 and H15 of 1.0–3.2 Hz indicated a planar W-type coupling⁵ and, hence, a cis relationship of these two hydrogens. The coupling constant between H5 and H16 (2.0–4.1 Hz) suggests that these protons are trans,⁶ whereas H15 and H16, which have a coupling constant of 10.0–12.4 Hz corresponding to a dihedral angle of ca. 0° , must be cis. The methyl protons of the ethyl group in **5** appear at an unusually high field (δ 0.48), and a Dreiding model (see also Figure 1) indicates that they lie within the shielding zone of the aromatic ring.⁷ This conformation also places the *N*-methyl group near the benzene ring of **5**, and, in agreement with this, it was found that irradiation of the NCH_3 proton signal caused a 5.3% nuclear Overhauser enhancement of H9. The interatomic separation of these groups is ca. 3.1 Å based on the configuration shown for **1**.

The stereostructure **1** (relative configuration) was finally confirmed by an X-ray analysis of koumine hydrobromide.⁸ A three-dimensional projection is shown in Figure 1. The novel skeleton of koumine places it biogenetically in the strychnos family of alkaloids,⁹ which includes the related oxindole gelsemine (**9**).¹⁰ However, the C_9 mevalonoid segment of **1**, presumably derived from secologanin, is attached to the tryptamine unit in a unique fashion.^{11,12}

Supplementary Material Available: ^1H and ^{13}C NMR data as well as proton coupling constants of koumine and its derivatives (4 pages). Ordering information is given on any current masthead page.

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(8) Crystals of koumine hydrobromide are orthorhombic and belong to the space group $D_2^7-P2_12_12_1$, $D_2^7-P2_12_12_1$, $a = 14.307$, $b = 12.053$, and $c = 9.862$ Å. Intensities were collected on a PW-1100 four-circle diffractometer by using $\text{Mo K}\alpha$ radiation; 1431 independent reflections with values 2.9 – 26.5° were measured. The position of the bromine atom was obtained from a three-dimensional Patterson synthesis and all nonhydrogen atoms were located in a one-cycle Fourier synthesis. After a second cycle with full matrix least-squares refinement, the R value was 0.13 (Yao, Z. H.; Wan, Z. L.; Liang, D. C., private communication).

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(12) An account of this work was presented at the Siao-American Symposium on the Chemistry of Natural Products, Shanghai, China, Oct. 27–31, 1980.

In Vivo Liquid Solution ENDOR and TRIPLE Resonance of Bacterial Photosynthetic Reaction Centers of *Rhodospseudomonas sphaeroides* R-26

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In the last few years ENDOR has proved a powerful tool for the investigation of primary reactants in bacterial photosynthesis.^{1–8} It has been concluded that a "special pair" of bacteriochlorophyll (BChl) upon excitation donates an electron to an electron-transport chain, leaving behind a cation radical accessible to ESR techniques.^{1,2,7,8} Together with primary (bacteriopheophytin = BPh) and secondary acceptors (ubiquinones), the special pair BChl is situated in the "reaction center" protein complex (RC), which can be isolated from several bacteria.^{7,9} The steric arrangement of the pigment molecules in the complex is strongly related to their photochemical function. Details of this arrangement are still unknown.^{7,10}

By liquid-solution ENDOR highest spectral resolution could be achieved with the isolated pigments, thereby yielding up to 10 proton and 4 nitrogen isotropic hfs couplings and a detailed picture of the spin density distribution of the monomeric radical ions of BChl *a* and BPh *a* in vitro.^{3–6} In vivo ENDOR studies of whole cells,¹ chromatophores,² or RC's^{11,12} have been carried out so far only at low temperatures, i.e., in solid solution. In these cases dipolar broadening masks all those interactions which do not belong to nuclei with relatively small hfs anisotropy, e.g., rotating methyl groups. Consequently, only a few broad ENDOR lines could be detected. Nevertheless, these investigations served as a strong support of the special pair model for the primary donor,^{1,2,7,8} which had originally been proposed from ESR data (see ref 8). This communication presents the first successful ENDOR experiments of RC's in aqueous solution at room temperature and demonstrates the possibility of studying biological samples at their correct physiological temperatures.

Reaction centers were isolated from *Rhodospseudomonas sphaeroides* R-26 by a modification of the method of Clayton and Wang.¹³ The crude reaction centers obtained after ammonium sulfate fractionation were purified on DEAE-cellulose (Whatman DE 52, 2.5-cm i.d. \times 20 cm for 1 μmol of reaction centers) and

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