High-Resolution Mass Spectrometry in Molecular Structure Studies. IV. Mechanistic Aspects of the Fragmentation of Widdrol[1,2]

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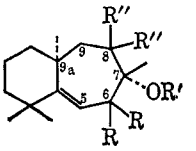
Abstract: The mass spectrum of the sesquiterpenol widdrol is dominated by the peak at *m/e* 151, corresponding to the charged species formed by loss of 71 mass units from the molecular ion. The mass spectra of a series of deuterium-labeled analogs and the complete high-resolution mass spectrum indicate that most of the M − 71 ions arise from a rearranged molecular ion, rather than from that of the original alicyclic alcohol. The presence of homoallylic unsaturation allows postulation of extensive rearrangement to a ketonic molecular ion.

In spite of their broad occurrence in nature, their chemical and biogenetic importance, their volatility, and the ample functional variation of basic skeletal moieties in this class of natural products, the sesqui- and also diterpenes have received little attention in the mass spectrometry literature thus far. A few complete low-resolution mass spectra and comparatively little mechanistic work toward their interpretation have been reported,[4] although the low-resolution mass spectrum of widdrol (Figure 1) and a suggestion for the composition and the origin of the dominating fragment[5] are among them.

This situation is clearly a result of the usual difficulties experienced in interpreting spectra of alicyclic compounds, even those of quite simple structure, without having at hand the necessary high-resolution data and supplemental information from labeled analogs. The demand for utilization of high-resolution techniques plus proper deuterium labeling as minimum requirements in the case of monoterpenes has only recently been emphasized by several authors.[6] Complete high-resolution mass spectra of the sesquiterpene lactone gaillardin and its derivatives have recently been presented by this laboratory in support of its structural elucidation.[7]

The structure and absolute configuration of widdrol (I) were established after extensive discussion in 1961.[5] Subsequently, in 1964, Dauben and Friedrich[8] elucidated the mechanism of conversion of thujopsene to widdrol in aqueous acidic media, the analogs 6,6-d_2-widdrol (III) (Figure 3) and 8,8-d_2-widdrol (IV) (Figure 4) being of primary importance in that context. The availability of these labeled compounds and that of widdrol-OD (II) (Figure 2) led to this mass spectral examination of the major modes of decomposition for this naturally occurring sesquiterpenol. Complete high-resolution data were determined for these compounds and presented in the format of heteroatomic plots[9] for ease of evaluation of labeling data.



I, R, R′, R″ = H
II, R, R″ = H; R′ = D
III, R = D; R′, R″ = H
IV, R, R′ = H; R″ = D

The Fragmentation of Widdrol

If widdrol is viewed as a cyclic alcohol, it would be expected to undergo the characteristic fragmentation outlined for alicyclic alcohols by Natalis[10] and substantiated by use of deuterium-labeled analogs for cyclopentanol[11] and cyclohexanol.[12] Thus, the molecular ion M_0 should suffer allylic bond scission α to the hydroxyl group, followed by transfer of an α-hydrogen and cleavage of the β,γ bond.

That this fragmentation sequence yielding ion a does occur to a certain extent is borne out by the high-resolution mass spectrum of widdrol (Figure 5), which shows that virtually all of the peak at *m/e* 71 arises from ions having the composition C_4H_7O. In the spectrum of 8,8-d_2-widdrol (IV) (Figure 4), most of the peak appears at *m/e* 72,[13] the corresponding ion retaining only one deuterium atom as expected. Similarly, in the low-resolution mass spectrum of widdrol-OD (II) (Figure

(1) For part III in the Berkeley series, see A. L. Burlingame, D. H. Smith, and W. J. Richter, *J. Am. Chem. Soc.*, in press. Financial support was provided in part by the National Aeronautics and Space Administration, Grant NsG 101.

(2) A portion of this material was presented at the 14th Annual Conference on Mass Spectrometry and Allied Topics, May 1966, Dallas, Texas.

(3) (a) Recipient of a fellowship from the American Association of University Women, 1965–1966. (b) F. Hoffmann-LaRoche & Company, Aktiengesellschaft, Basel, Switzerland.

(4) R. I. Reed in "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press Inc., New York, N. Y., 1963, pp 672–676; R. T. Aplin and T. G. Halsall, unpublished work in H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day, Inc., San Francisco, Calif., 1964, pp 151–153; R. Hodges, E. P. White, and J. S. Shannon, *Tetrahedron Letters*, 371 (1964); G. Lukas, J. C. N. Ma, J. A. McCloskey, and R. E. Wolff, *Tetrahedron*, 20, 1789 (1964); G. L. K. Hunter and W. B. Brogden, *J. Org. Chem.*, **29**, 982 (1964); N. Wasuda and T. Tsuchiya, 13th Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, Mo., 1965, p 455; D. G. B. Boocock and E. S. Waight, *Chem. Commun.*, 90 (1966).

(5) C. Enzell, *Acta Chem. Scand.*, **16**, 1553 (1962).

(6) D. S. Weinberg and C. Djerassi, *J. Org. Chem.*, **31**, 115 (1966); A. F. Thomas and B. Willhalm, *J. Chem. Soc., Phys. Org.*, 219 (1966).

(7) S. M. Kupchan, J. M. Cassady, J. E. Kelsey, H. K. Schnoes, D. H. Smith, and A. L. Burlingame, *J. Am. Chem. Soc.*, **88**, 5292 (1966).

(8) W. G. Dauben and L. E. Friedrich, *Tetrahedron Letters*, 2675 (1964).

(9) A. L. Burlingame and D. H. Smith, in preparation.

(10) P. Natalis, *Bull. Soc. Roy. Sci. Liege*, **31**, 790 (1962).

(11) P. Natalis, *Bull. Soc. Chim. Belges*, **69**, 224 (1960).

(12) H. Budzikiewicz, Z. Pelah, and C. Djerassi, *Monatsh. Chem.*, **95**, 158 (1964); C. G. McDonald, J. S. Shannon, and G. Sugowdz, *Tetrahedron Letters*, 807 (1963).

(13) All intensities of peaks discussed are corrected for isotopic purity.

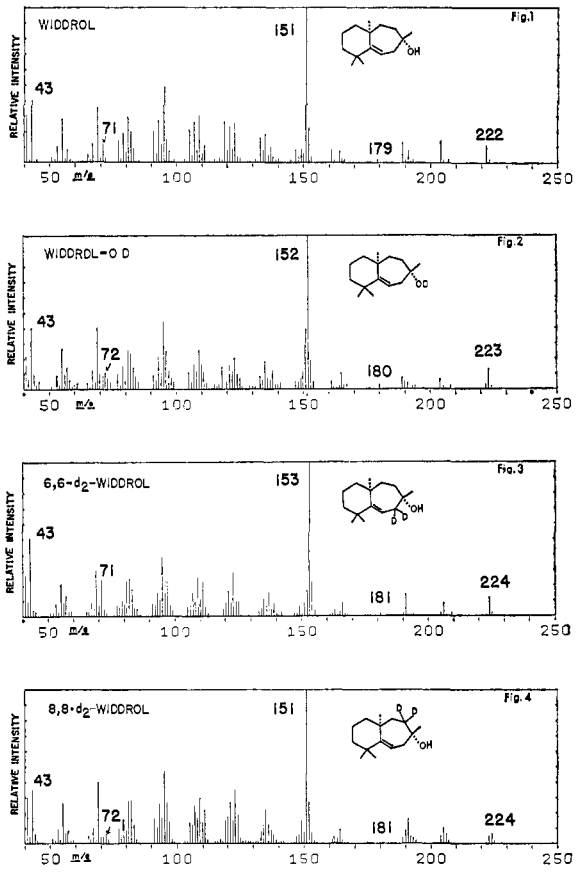
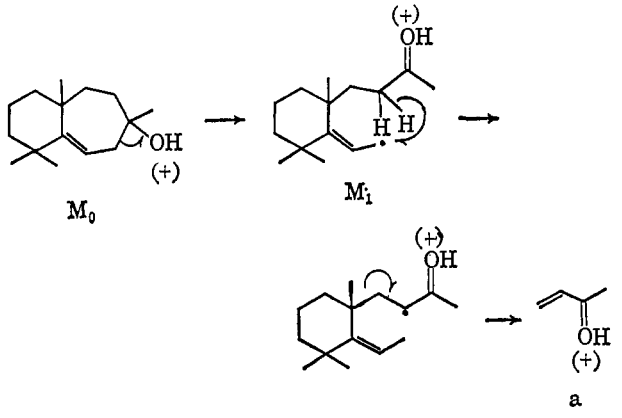
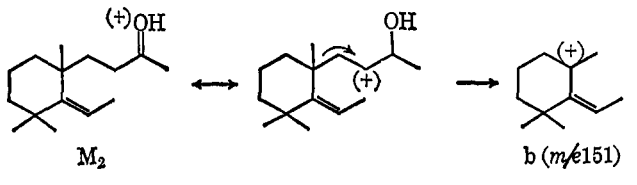


Figure 1. Mass spectrum of widdrol (I).
Figure 2. Mass spectrum of widdrol-OD (II).
Figure 3. Mass spectrum of 6,6-d_2-widdrol (III).
Figure 4. Mass spectrum of 8,8-d_2-widdrol (IV).

2), at least 70% of the peak at *m/e* 71 is shifted to *m/e* 72 owing to predominant retention of the hydroxyl hydrogen atom.[13]



It should be noted, in addition, that some of the charge seems to be transferred to the hydrocarbon moiety in the final step of this fragmentation sequence, leading to ion b. The precursor ion for both modes of final cleavage, namely, the rearranged molecular ion M_2, can be viewed as a "new" enolic molecular ion.



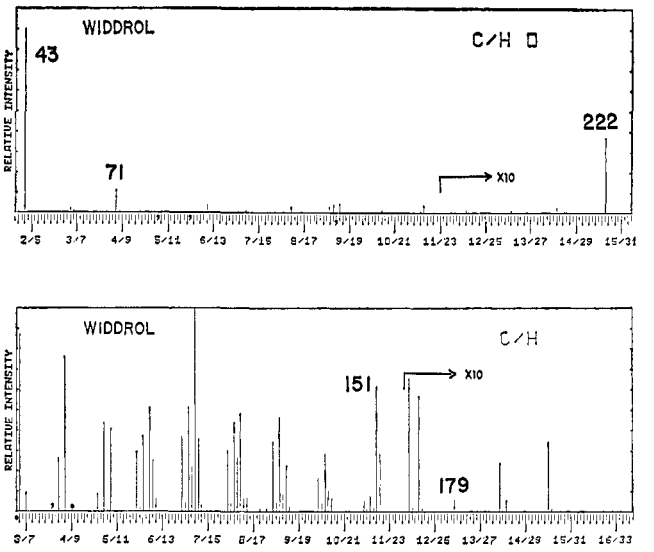
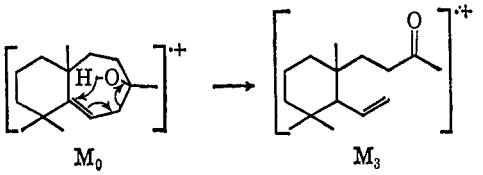


Figure 5. High resolution mass spectrum of widdrol (I).

Such heterolytic cleavage of the 9,9a bond accounts for only a minor portion of the total ion current at *m/e* 151. Support for a second process contributing to the $C_{11}H_{19}$ ions is shown by noting that (a) 15% of the original peak intensity remains at *m/e* 151 in widdrol-OD, and (b) a comparable fraction of the $C_{11}H_{19}$ ions shifts to *m/e* 152 in 8,8-d_2-widdrol (Figure 4).

As evident from the shift of the major part of the peak at *m/e* 151 to 152 in the spectrum of compound II (Figure 2), another and much more important mode of fragmentation of the skeleton must be operative in addition to that leading to ion b. The sequence of processes involved seems to be triggered by the presence of the homoallylic bond, since there is no evidence for an analogous sequence in the fragmentation of saturated alicyclic alcohols.

The interpretation presented for the genesis of this main component of the $C_{11}H_{19}$ ion current includes again as the initial step a rearrangement of the original molecular ion M_0 to an "open" isomeric species M_3, which represents a "ketonic" ion in contrast to the "enolic" ion M_2. Several mechanistic pathways may be postulated for this rearrangement. One mechanistic possibility ($M_0 \rightarrow M_3$) would depict transfer of the hydroxyl hydrogen to the double bond in M_0 *via* a six-membered transition state with rupture of the B ring resulting in the generation of a "methyl ketonic" molecular ion, M_3.

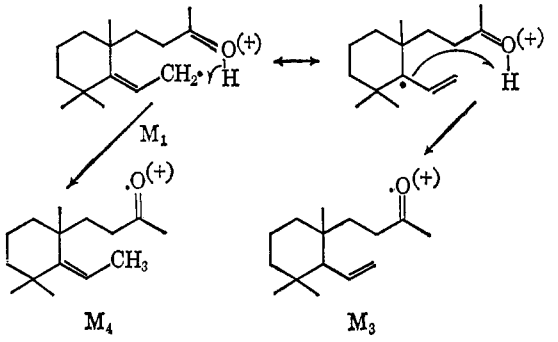


Such a hydrogen transfer with double-bond migration can be regarded as a cyclic analog of the frequently observed McLafferty rearrangement of open-chain olefins or carbonyl compounds containing γ-hydrogen atoms attached to carbon. Djerassi, *et al.*,[14] have suggested from their work on McLafferty rearrangements in steroidal ketones a 1.8-A maximum allowable distance

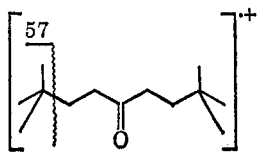
(14) C. Djerassi and L. Tokes, *J. Am. Chem. Soc.*, 88, 536 (1966), and references therein.

between carbonyl oxygen and the hydrogen atom transferred. Although the steroid model is not strictly analogous, a comfortable conformation of the atoms concerned within 1.8 A is easily possible for widdrol, as estimated from Dreiding models.

A mechanistic alternative involves initial α-allylic cleavage with subsequent abstraction of the hydroxyl hydrogen *via* a seven- or nine-membered transition state. Again, an open ketonic molecular ion, *e.g.*, M_3 or M_4, would result. That a predominant preference exists in the case of M_1 for the abstraction of the hydroxyl hydrogen rather than the activated α-hydrogens, as is the dominant process in the alicyclic alcohol model,[10-12] would not be expected, and the driving force is unclear. Of course, generation of an allylically stabilized radical leads to a less reactive radical site and, as a consequence, higher selectivity rendering preferred abstraction from conformationally less but energetically more favorable sites could result. In contrast to the foregoing discussion, conceptual preference for the hydroxyl hydrogen over that of C-7 methyl hydrogens utilizing a McLafferty-type mechanism is reasonable.

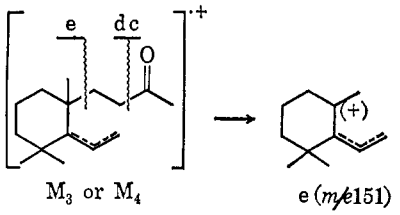


Assuming the formation of such a rearranged molecular ionic species, *e.g.*, M_3 or M_4, its subsequent modes of fragmentation will then be governed by its methyl ketonic nature. α Cleavage produces ion c (C_2H_3O), as expected, from a methyl ketone, which accounts for nearly all of the abundant peak at *m/e* 43. The corresponding C_2H_3O ions formed from the three labeled derivatives contain no deuterium atoms. A minor portion of the charge is carried by the complementary fragment d (*m/e* 179). This hydrocarbon fragment acquires one deuterium atom in the spectrum of widdrol-OD (Figure 2), two deuterium atoms in 6,6-d_2-widdrol (Figure 3), and also two atoms in 8,8-d_2-widdrol (Figure 4).[13] The classical McLafferty ketone rearrangement of M_3 or M_4 is *a priori* inhibited since the position γ to the carbonyl function is quaternary. A datum from the literature[15] reveals that the major contribution to the total ionization of a model γ-quaternary ketone, *cf.* 2,2,8,8-tetramethylnonan-5-one (V), is borne by the quaternary hydrocarbon moiety upon cleavage of the β,γ bond.



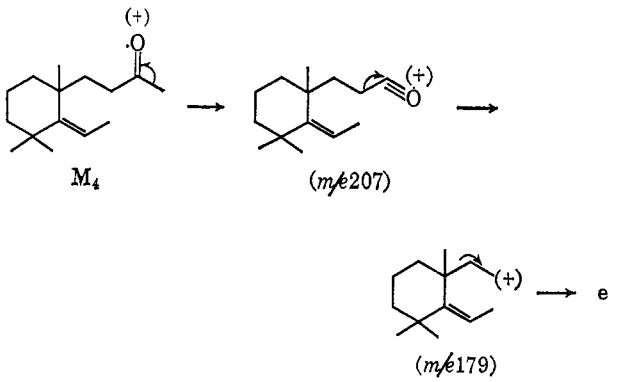
V

Formally, this is the exact mechanistic model which is invoked to explain the major contribution to the peak at nominal *m/e* 151, which dominates the fragmentation pattern of widdrol M_3 or $M_4 \rightarrow$ e.



Data from the deuterium-labeled derivatives quantitatively support this discussion, *e.g.*, (a) in widdrol-OD (Figure 2), 85%[13] of the peak at *m/e* 151 shifts to *m/e* 152; (b) in 6,6-d_2-widdrol (Figure 3), an even more significant portion shifts to *m/e* 153; and (c) in 8,8-d_2-widdrol (Figure 4), the peak at *m/e* 151 remains unshifted, since C-8 and attached deuterium atoms are eliminated in the fragmentation.

This second phase of the fragmentation of the original molecular ion, namely, the final decomposition of the rearranged "ketonic" species to the fragment of mass 151, may, as formulated, proceed *via* a direct cleavage as a one-step process or may comprise a sequence of several steps. Considering the latter case, a sequential loss of methyl radical, carbon monoxide, and ethylene from any of the ketonic intermediates would give ion e as well.



There are, however, no metastable peaks in the spectrum of widdrol permitting a decision between these two alternatives. The model ketone V does exhibit a metastable peak for the second step, namely, the loss of carbon monoxide from the acylium ion.[16] The M − 15 and M − 43 peaks, *e.g.*, *m/e* 207 and 179, respectively, in the spectrum of widdrol could be interpreted as being intermediates of such a sequential fragmentation.

Several ions of abundance comparable to that of the molecular ion can be defined with regard to their structural origin. The first ion (f) occurring at *m/e* 164 (M − 58, $C_{12}H_{20}$) in the natural product shifts unexpectedly to *m/e* 165 in widdrol-OD and to *m/e* 166 in 6,6-d_2-widdrol and remains unshifted in 8,8-d_2-widdrol. Since the hydroxyl hydrogen is retained in the fragment under discussion, one is required to postulate the occurrence of a "reciprocal" double hydrogen transfer during the lifetime of the molecular ion. This behavior is analogous to that of ion e and would suggest, therefore,

(15) R. Arndt and C. Djerassi, *Chem. Commun.*, 578 (1965).

(16) Professor C. Djerassi, Stanford University, private communication.

a common mechanistic genesis. The neutral species eliminated is of composition C_2H_6O and would be consistent with loss of a molecule of acetone from ions M_3 or M_4. Positional origin for the hydrogen transferred back to the carbonyl group cannot be deduced from these data, but it is assumed that a five- or seven-membered transition state is involved.

Thus, fragmentation can be distinguished in the mass spectrum of widdrol from "alcohol" as well as "ketone" molecular ions, which both produce daughter ions of the same *exact mass* in their major fragmentation modes. In one case, the hydroxyl hydrogen is transferred, and the charge remains primarily on the hydrocarbon moiety e. In the other, a ring hydrogen is transferred, and most of the charge remains on the oxygen-containing fragment a. Such rearrangement of molecular ionic species prior to fragmentation is not unique to the β,γ-unsaturated alcohol, widdrol.[17] Similar observations and suggestions have been reported in at least six earlier papers, *e.g.*, on 2-phenylethanol[18] and derivatives,[19] on derivatives of 3-buten-1-ol,[19,20] on β-hydroxy esters,[21] on several hydroxy alkaloids,[22] and on 19-hydroxy steroids.[23] The thermal equivalent of such a rearrangement is known as well, *e.g.*, in β-hydroxy olefins[24] and β-hydroxy esters.[21]

(17) The high-resolution spectra of isopulegol, terpen-4-ol, and their OD analogs obtained in this laboratory indicate that these homoallylic alcohols also rearrange to carbonyl ions under electron impact.

(18) A. Gilpin, *J. Chem. Phys.*, **28**, 521 (1957).

(19) H. E. Audier, H. Felkin, M. Fetizon, and W. Vetter, *Bull. Soc. Chim. France*, 3236 (1964).

(20) J. W. Cornforth, R. H. Cornforth, G. Popjak, and L. Yengoyan, *J. Biol. Chem.*, 3970 (1966).

(21) A. H. Etemadi, *Bull. Soc. Chim. France*, 1537 (1964).

(22) C. Djerassi, H. Budzikiewicz, R. J. Owellen, J. M. Wilson, W. G. Kump, D. J. LeCount, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **46**, 742 (1963); M. Pinar, W. V. Philipsborn, W. Vetter, and H. Schmid, *ibid.*, **45**, 2260 (1962).

(23) S. H. Eggers, *Tetrahedron Letters*, 733 (1965).

(24) R. T. Arnold and G. Smolinsky, *J. Am. Chem. Soc.*, **82**, 4918 (1960); R. T. Arnold and G. Smolinsky, *ibid.*, **81**, 6443 (1959).

(25) M. Senn, W. J. Richter, and A. L. Burlingame, *ibid.*, **87**, 680 (1965).

(26) F. C. Walls and A. L. Burlingame, Abstracts of Papers Presented at the 4th Annual Meeting of the Society for Applied Spectroscopy, Denver, Colo., Sept 1–5, 1965.

Experimental Section

Widdrol-OD (II) was obtained by exchanging a sample of widdrol on a D_2O-treated vpc column[25] and co-inserting it into the mass spectrometer with a microliter of heavy water. Its isotopic purity was calculated from the spectrum to be 83% d_1 and 17% d_0. The isotopic purity of 6,6-d_2-widdrol was determined as 93% d_2 and 7% d_1, and that of 8,8-d_2-widdrol as 43% d_2 and 43% d_1.

The low-resolution mass spectra of widdrol, widdrol-OD, 6,6-d_2-widdrol, and 8,8-d_2-widdrol were obtained on a modified[26] CEC 21-103C mass spectrometer (inlet system 100°, ion source 180°, ionizing energy 70 ev). The high-resolution mass spectrum of widdrol was obtained on a CEC 21-110B mass spectrometer operating with the inlet system at 180° and the ion source at 200°. In the spectra presented (Figure 5), the masses are plotted in methylene units.[9] On the abscissa, each major division marker corresponds to the saturated ion, *e.g.*, C_nH_{2n+1}, with the number of carbon atoms given in the top row of figures and the number of hydrogen atoms indicated in the bottom row. There are 14 units between each major division, and the number of hydrogen atoms of an unsaturated or cyclic ion is obtained simply by determining the difference from the position of the next higher saturated ion.

Acknowledgment. The authors are indebted to Professor W. G. Dauben for kindly providing the deuterated widdrol analogs II and III and to L. E. Friedrich for valuable discussions.

Structures of the Indole Alkaloids Kopsingine and Kopsaporine

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Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, and from the Department of Chemistry, University of Singapore, Singapore. *Received November 4, 1966*

Abstract: Structures **4** and **5**, respectively, were assigned to kopsingine and kopsaporine, the two major alkaloids of *Kopsia singapurensis*. The carbon skeleton of kopsingine was confirmed by conversion to 17-methoxy-N-methylaspidofractinine, and the assignment of functional groups was based on the mass spectra of kopsingine and numerous derivatives. Kopsaporine was shown to be demethoxykopsingine by a comparison of its mass and nmr spectra with data from kopsingine.

Kopsingine, kopsaporine, and kopsingarine have been reported to occur as the major alkaloidal constituents of *Kopsia singapurensis*.[2] These alkaloids account for 3% of the dried leaves of this Malayan species, with kopsingine being obtained in 2.2% yield. It will be shown that these compounds are structurally related to several *Kopsia* alkaloids, but their presence has not been detected in other *Kopsia* species.

Earlier work[2] on the structure of kopsingine established a molecular composition of $C_{24}H_{28}N_2O_7$, which has now been confirmed by an accurate mass determination (calcd 456.1896, found 456.1860). The ultraviolet spectrum of kopsingine [λ_{max}^{MeOH} 217 mμ (log ε 4.56), 253 (4.04), 282 (3.38), 288 (3.36)] is similar to reported N-carbomethoxyindoline spectra [pleiocarpine (**1**)[3,4] λ_{max} 207 mμ (log ε 4.49), 246 (4.20), 283 (3.51), 290

(1) (a) Massachusetts Institute of Technology, Cambridge, Mass. (b) University of Singapore, Singapore.

(2) (a) A. K. Kiang and R. D. Amarasingham, *Proc. Symp. Phytochem., Kuala Lumpur*, 165 (1957); *Chem. Abstr.*, **53**, 14131 (1959); (b) R. D. Amarasingham, M.S. Thesis, University of Malaya, 1961.

(3) W. G. Kump and H. Schmid, *Helv. Chim. Acta*, **44**, 1503 (1961).

(4) W. G. Kump, D. J. LeCount, A. R. Battersby, and H. Schmid, *ibid.*, **45**, 854 (1962).