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).

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.

(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.

Structures of the Indole Alkaloids Kopsingine and Kopsaporine

David W. Thomas,[1a] K. Biemann,[1a] A. K. Kiang,[1b] and R. D. Amarasingham[1b]

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

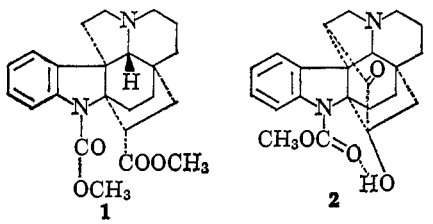
(1) (a) Massachusetts Institute of Technology, Cambridge, Mass. (b) University of Singapore, Singapore.
(2) (a) A. K. Kiang and R. D. Amarasingham, *Proc. Symp. Phytochem., Kuala Lumpur*, 165 (1957); *Chem. Abstr.*, **53**, 14131 (1959); (b) R. D. Amarasingham, M.S. Thesis, University of Malaya, 1961.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

(3.48); kospine (**2**)[5] λ_{max} 240 mμ (log ε 4.08), 278 (3.37), 285–286 (3.35)].

The presence of a hydrogen-bonded urethan group could be deduced from absorption in the infrared at 1670 cm^{-1}. This is analogous to the kopsine (**2**) infrared absorption at 1679 cm^{-1},[5] whereas a free urethan [*e.g.*, pleiocarpine (**1**)] absorbs near 1705 cm^{-1}.[3] The infrared spectrum of kopsingine shows additional absorption at 1740 cm^{-1}, which was attributed to an ester function. These two carbomethoxyl groups could be chemically verified by their reduction with lithium aluminum hydride, a reaction which will be discussed in greater detail later.

Conventional analysis had indicated the presence of three methoxyl groups,[2] also apparent in the nmr spectrum of kopsingine at 3.77, 3.79, and 3.82 ppm. Two of these were accounted for by the carbomethoxyl groups. The third had the chemical shift of an aromatic methoxyl, an assignment which is consistent with the observation of only three aromatic hydrogens in the nmr spectrum (6.6–7.2 ppm) and which may explain the small differences between the ultraviolet spectra of kopsingine and the unsubstituted reference compounds cited above.

The nmr spectrum of kopsingine showed absorption of two vinyl protons at 5.62 and 5.95 ppm. These signals were absent in the spectrum of dihydrokopsingine (mol wt, 458), obtained by catalytic hydrogenation of kopsingine. The vinyl protons were coupled to each other (J = 10 cps), and one of these protons (5.95 ppm) was further split by two adjacent nonequivalent protons (J = 4 and 2 cps), the latter appearing as a multiplet at 3.3 ppm. These observations are nearly identical with data on the *Vinca* alkaloid vindoline, to which structure **3** had been assigned.[6]

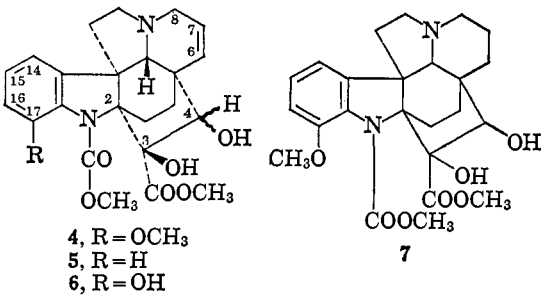


Kopsingine contained two active hydrogen atoms, as demonstrated by conventional analysis as well as by the incorporation of two deuterium atoms when introduced into the mass spectrometer in the presence of deuterium oxide. The remaining two oxygens of kopsingine, thus far unidentified, may therefore be accounted for as hydroxyl groups. Only one hydroxyl was reactive, since a monoacetate could readily be prepared (mol wt, 498; nmr 1.92 ppm, 3 H). It was also apparent that this hydroxyl was secondary, as the nmr spectrum of kopsingine acetate showed a one-proton signal at 5.15 ppm (a doublet due to long-range coupling, J = 2 cps) from the proton on the carbon bearing the acetate group. The infrared absorption of the acetate at 1670 cm^{-1} indicated that the second hydroxyl, probably tertiary, was responsible for the hydrogen bonding of the urethan.

Because of the striking similarity of the data for kopsingine and vindoline (**3**), structure **4** for kopsingine was proposed as a working hypothesis. Confirmation of this structure, including the exact position of aromatic substitution and the relative stereochemistry, resulted from data discussed in the remainder of this paper.[7] An alternative location of the double bond at C-7–C-8 was not consistent with the nmr spectrum of kopsingine, which indicated that the methylene group (3.3 ppm) next to the double bond was further deshielded by an adjacent nitrogen atom. A double bond at C-6–C-7 has also been found in vindolinine,[8] tabersonine,[9] vindoline,[6] and venalstonine.[10]

Structure **4** is supported by the mass spectra of kopsingine and dihydrokopsingine (**7**). These spectra however are not very characteristic and involve mainly the losses of elements of the C-3–C-4 bridge.

Conclusive evidence for the proposed structure for kopsingine was obtained by the chemical transformations below.



The Carbon Skeleton and Its C-3 Substituents. A crucial piece of evidence is the conversion of kopsingine to a compound representing a known ring system by a sequence of a few uncomplicated reactions outlined in Scheme I.

On treatment with *p*-toluenesulfonyl chloride, dihydrokopsingine (**7**) formed a monotosylate **9** (nmr 2.45 ppm, 3 H). Reduction of the tosylate with lithium aluminum hydride yielded two major products: (a) a diol **10** (mol wt, 370) formed by displacement of the tosylate ester, and (b) a triol **11** (mol wt, 386) formed by cleavage of the S–O bond. The number of hydroxyl groups in each derivative was verified by exchange with deuterium oxide which resulted in a shift of the molecular ions of these compounds to m/e 372 and 389, respectively.

(5) T. R. Govindachari, B. R. Pai, S. Rajappa, N. Viswanathan, W. G. Kump, K. Nagarajan, and H. Schmid, *Helv. Chim. Acta*, **45**, 1146 (1962); **46**, 572 (1963).

(6) (a) M. Gorman, N. Neuss, and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962); (b) J. W. Moncrief and W. N. Lipscomb, *Acta Cryst.*, **21**, 322 (1966).

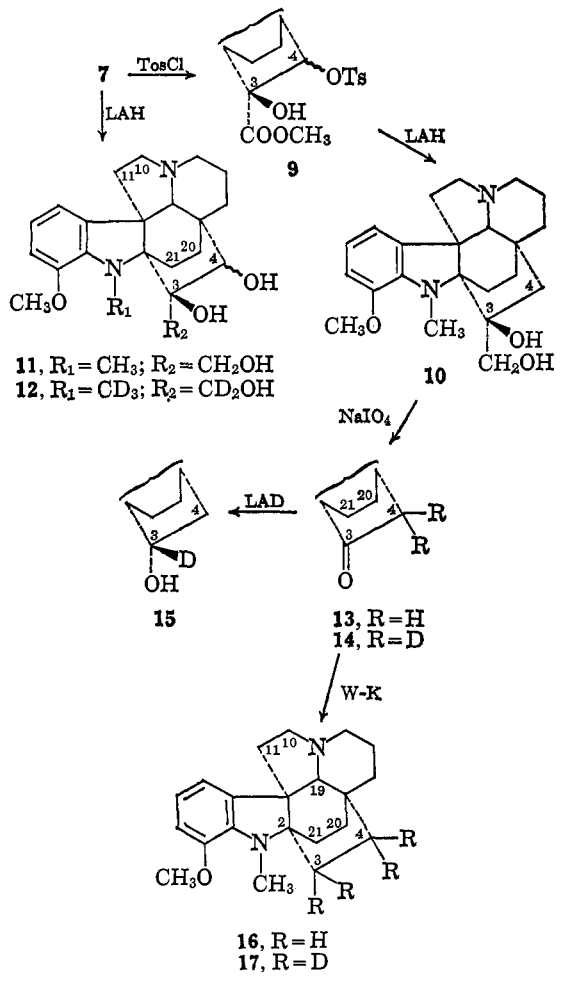
(7) To simplify discussion of the data and arguments, the substituents are always shown at C-3 and C-4 (see structure **4**). It should be kept in mind that most of the arguments would hold equally well if they were on C-21 and C-20. The correctness of the former representation will be proven later in this paper.

(8) C. Djerassi, S. E. Flores, H. Budzikiewicz, J. M. Wilson, L. J. Durham, J. LeMen, M.-M. Janot, M. Plat, M. Gorman, and N. Neuss, *Proc. Natl. Acad. Sci. U. S.*, **48**, 113 (1962).

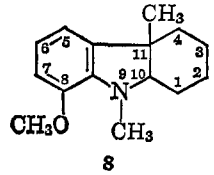
(9) M. Platt, J. LeMen, M.-M. Janot, J. M. Wilson, H. Budzikiewicz, L. J. Durham, Y. Nakagawa, and C. Djerassi, *Tetrahedron Letters*, 271 (1962).

(10) B. Das, K. Biemann, A. Chatterjee, A. B. Ray, and P. L. Majumder, *ibid.*, 2239 (1965).

Scheme I



The triol **11** was also obtained in the hydride reduction of dihydrokopsingine. Conversion of the urethan to an N-methyl group was apparent from the presence of an appropriate signal at 3.12 ppm in the nmr spectrum, and the incorporation of five deuterium atoms (**12**, mol wt, 391) with lithium aluminum deuteride confirmed the nature of this reaction. The ultraviolet spectrum of the triol **11** [λ_{max}^{MeOH} 216 mμ (log ϵ 4.40), 260 (3.87), 295 (3.34)], as well as the diol and all other N-methyl derivatives, was similar in both peak positions and shape of curve to spectra of N-methylindolines substituted at the position *ortho* to the nitrogen. A suitable model is 1,2,3,4,10,11-hexahydro-8-methoxy-9,11-dimethylcarbazole (**8**) which exhibits λ_{max} 253 mμ (log ϵ 3.86) and 293 (3.18).[11] This observation established the aromatic methoxyl group of



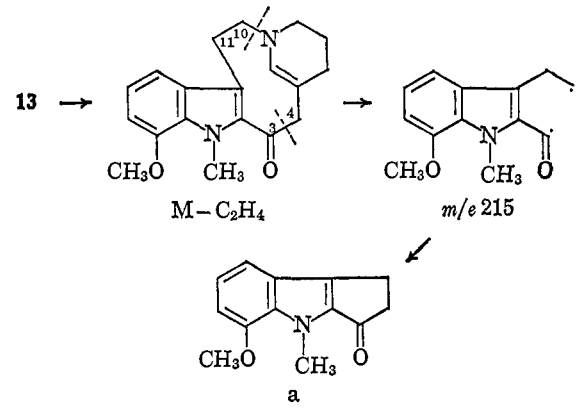
8

kopsingine at C-17 (see structure **4** for numbering system).

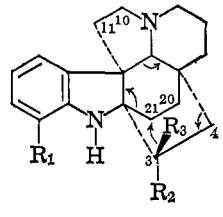
The above-mentioned diol **10** proved to be a 1,2 diol, as it reacted with sodium periodate to give the ketone **13** (mol wt, 338; γ_{max} 1720 cm^{-1}). The latter gave a monodeuterated alcohol (**15**, mol wt, 341) on reduction with lithium aluminum deuteride, and the compound **16** (17-methoxy-N-methylaspidofractinine) on Wolff–Kishner reduction. The evidence on which these assignments are based will be outlined below.

The mass spectrum of the diol **10** (mol wt, 370) was consistent with the structure proposed. In particular, the most intense peak at *m/e* 110 is characteristic[12,13] of alkaloids of the aspidofractinine skeleton (**18**) which have a hydrogen-bearing substituent (such as a hydroxyl group) at C-3, but lack functional groups at C-4.

Ketone **13** gave a mass spectrum which was consistent with the proposed structure. The most abundant fragment of this spectrum was at *m/e* 215, and did not contain C-4 (*m/e* 215 from **14** rather than *m/e* 217). It seemed likely that homolytic cleavage at C-3–C-4 and C-10–N_b of an M − C_2H_4 ion could produce an ion of mass 215, which then might cyclize to form the fragment a, as diagrammed below.



The position of the carbonyl group in the ketone **13**, and hence of the tertiary hydroxyl and carbomethoxyl functions of kopsingine, was confirmed by conversion to the deuterated alcohol **15**. The mass spectrum of the latter is analogous to that of compound **21**[12,13] (except the difference of 14 mass units in some of the peaks) with the most intense peak at *m/e* 110 (characteristic of a C-3 alcohol) and the second major peak at *m/e* 125. The occurrence of the latter peak at *m/e* 125 rather than 124 conclusively proved that deuterium was introduced at C-3, since only the substituent R_3 in **21** was found to be transferred in the formation of this ion.[12,13]



18, $R_1 = R_2 = R_3 = H$
19, $R_1 = OCH_3$; $R_2 = R_3 = H$
20, $R_1 = OCH_3$; $R_2 = OH$; $R_3 = H$
21, $R_1 = OCH_3$; $R_2 = OH$; $R_3 = D$

(11) M. F. Millson and R. Robinson, *J. Chem. Soc.*, 3362 (1955).

(12) 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).

(13) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 1, Holden-Day, Inc., San Francisco, Calif., 1964.

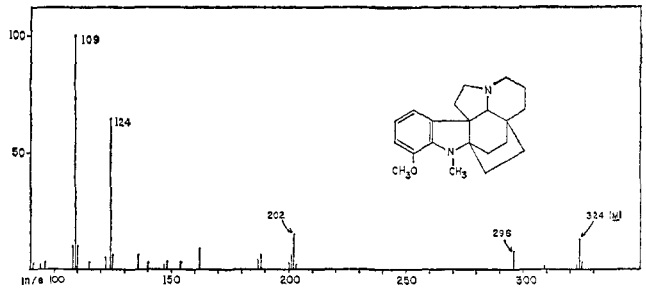


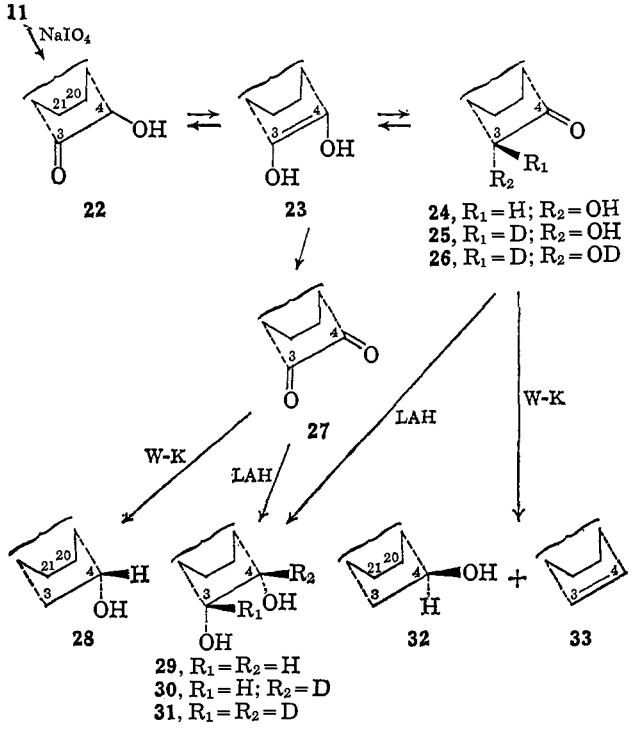
Figure 1. Mass spectrum of 17-methoxy-N-methylaspidofractinine (**16**).

The relative stereochemistry at C-3 of kopsingine follows from the observation that only the epimer indicated by structure **4** brings the C-3 hydroxyl group and the urethan carbonyl function close enough to form a hydrogen bond.

The most conclusive verification of the skeleton of kopsingine was provided by a mass spectrum of the reduction product **16** (Figure 1), which showed all the peaks characteristic of the aspidofractinine skeleton. This spectrum is very similar to the published spectra of aspidofractinine itself (**18**) and 17-methoxyaspidofractinine (**19**),[13] except for the shifts of all ions containing aromatic substituents, occurring at *m/e* 324 (M), 296, and 202, and the minor differences in relative peak intensities which are to be expected.

Location of the C-4 Hydroxyl Function. Another part of structure **4** which required confirmation was the location of the secondary hydroxyl, suggested to be at C-4, and this was achieved through the reactions of the triol **11** (Scheme II).

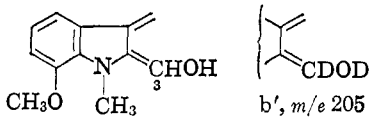
Scheme II



Treatment of triol **11** with sodium periodate gave a mixture of products, the formation of which is unusual in many respects. It was immediately apparent that cleavage at C-3–C-4 had not occurred, probably because the intermediate five-membered periodate ring might interfere with either the 20–21 or the 10–11 bridge. However, the primary alcohol function in **11** was readily lost, resulting in an α-diketone **27**, an α-ketol **24**, and a comparatively small quantity of an unstable compound thought to be the enediol **23**. The properties of these products thus require the presence of a secondary hydroxyl group at a carbon atom next to that bearing the tertiary hydroxyl of kopsingine.

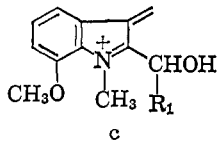
The diketone **27** (mol wt, 352; γ_{max} 1720, 1755 cm^{-1}) was isolated as orange needles, mp 184–187°. It resulted either from air oxidation of the ketol **24** or, more likely, from the enediol **23**, since the latter was observed to give nearly equal amounts of **24** and **27** on standing.

The ketol (mol wt, 354; γ_{max} 1715 cm^{-1}) was originally thought to have structure **22**, with a ketone function at C-3. However, its mass spectrum had a major peak at *m/e* 124, a result which was shown earlier to require a hydrogen atom at C-3.[12,13] Furthermore, the monodeuterated derivative **25**, obtained by base-catalyzed exchange with deuterium oxide, produced a shift of the major part of this peak to *m/e* 125. In addition, a significant ion of *m/e* 203 from the ketol was shifted to *m/e* 204 in the spectrum of **25** and to *m/e* 205 when deuterium was also incorporated in the hydroxyl group (**26**). The fragment at *m/e* 203 must have structure b, containing C-3 as well as the oxygen function at that carbon. The two deuterium atoms can only be incorporated in this fragment if the C-3 function is a secondary alcohol, and the ketol therefore is best represented by structure **24**. Isolation of the ketol in this form suggests that the isomerization of **22** to **24** takes place readily *via* the enediol intermediate, which relieves the steric crowding of a tetrahedral carbon at C-4. The preference for a trigonal carbon at C-4 is also indicated from an examination of Dreiding models.



b, *m/e* 203 b′, *m/e* 205

The diketone and the ketol were directly correlated by hydride reduction of each to the same diol **29**. Use of deuteride gave two labeled diols, **30** and **31**. The mass spectra of these gave additional confirmation of the structure **24** for the ketol. In particular, the peaks at *m/e* 124 and 204 (c) in the spectrum of **29** were shifted to *m/e* 125 and 205 only in the spectrum of the diol-d_2 **31** derived from the diketone, which implied that the deuterium atom in **30**, obtained from the ketol, was located at C-4.



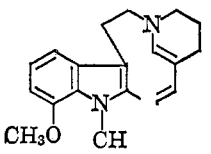
c

The diketone **27** was originally considered a suitable starting material for the preparation of compound **16** by Wolff–Kishner reduction; however, the single product obtained proved instead to be the alcohol **28**. Further investigation of this reaction showed that only a monohydrazone had formed, and the remaining

ketone function at C-4 subsequently became reduced (presumably from the less hindered side) by the alkoxide during pyrolysis. These results again point to an unusual environment of C-4, also noticed in the isomerization of the α-ketols, which could perhaps also be related to the failure of the C-3–C-4 periodate cleavage of the triol **11**.

The reluctance of the C-4 ketone function to form a hydrazone may also account for the formation of alcohol **32** by Wolff–Kishner reduction of **24**. The mass spectrum of **32** was distinctly different from that of the isomeric alcohol **15** (hydrogen instead of deuterium at C-3), the expected reduction product. In fact, the spectra of **28** and **32** were strikingly similar (peaks at *m/e* 188, 125, and 124) with only small differences in relative peak intensities, reflecting the epimerization at C-4. If a C-4 carbonyl function is unreactive toward hydrazine, the ketol **24** could have been isomerized to form the hydrazone of **22**. The product could then undergo normal Wolff–Kishner reduction to the less hindered C-4 epimer **32**.

A second major product of the Wolff–Kishner reduction of the ketol **24** arose by an abnormal reaction, which is also occasionally observed with α-substituted hydrazones,[14] in which the α substituent (a hydroxyl group in this case) is lost to give an olefin **33**. The mass spectrum of this compound shows a very pronounced loss of ethylene, facilitated by the extended conjugation of the resulting ion d (*m/e* 294). The nmr spectrum of **33** exhibited two doublets for the resonance of two olefinic protons (6.08 and 6.45 ppm, J = 9 cps), which confirmed the assignment of an isolated double bond on one of the ethylene bridges.



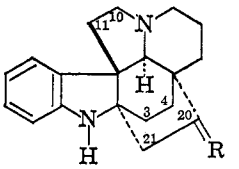
d

Catalytic reduction of the olefin **33** completed an independent route to the previously derived 17-methoxy-N-methylaspidofractinine (**16**), thereby verifying all features of the structure of kopsingine, with the exception of the still uncertain relative stereochemistry at C-4.

Substituents at C-3–C-4 *vs.* **C-21–C-20**. The remaining possibility that the substituents are located at C-21–C-20 is eliminated by the following observations.

It had been noticed in substituted derivatives of aspidofractinine that the unsubstituted bridge (that which is *trans* to the tryptophan bridge) was always lost in the formation of the M − 28 ion.[12,13] This feature, which is also evident in the mass spectra of many kopsingine derivatives, was tentatively taken as an indication that here also the substituents are at the same (*cis*) bridge. The preparation of the deuterated derivative **17** strengthened this hypothesis. Base-catalyzed exchange of ketone **13** with deuterium oxide gave the labeled derivative **14**, which was reduced by a Wolff–Kishner reaction in methanol-*d*. The mass spectrum of the product **17** had peaks at *m/e* 328 (M), 300, 204, 190, 125, and 111, indicating the exclusive loss of a completely unlabeled bridge. It will be shown by comparison with compounds known to be oxygenated at C-20 that in kopsingine the substituents are at C-3 and C-4, *i.e.*, at the *cis* bridge. As a corollary, the spectrum of **17** implies that the preferred loss of one of the two bridges is controlled by its stereochemistry and not by the presence or absence of substituents.[15]

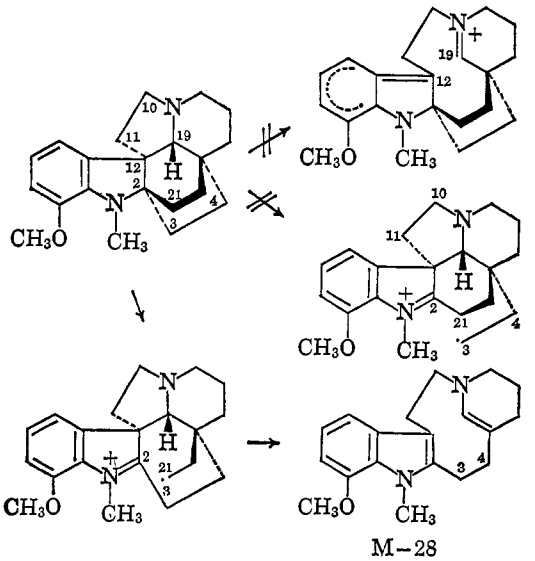
A comparison of the mass spectra of alcohols **28** and **32** [*m/e* 340 (M), 312 (M − 28), 188, 125, 124] with that of compound **34** [*m/e* 296 (M), 252 (M − 44), 140, 109[16]] provided additional evidence for the presence of the functional groups of kopsingine at the C-3–C-4 bridge. In each case, only the bridge *trans* to the tryptophan bridge was lost, even though this was the substituted bridge in **34** (M − 44) and the unsubstituted bridge in **28** and **32** (M − 28). Because of the significant implications of this result, it was necessary to make very certain that the pair **28** and **32** have the substituents indeed on the other bridge in comparison with **34**. Three of the four possible alcohols with the hydroxyl groups on the same bridge (**15**, **28**, and **32**) have been obtained from kopsingine. The possibility that **23** could be the fourth, and that **28** and **32** could be C-21



34, R = H; OH
35, R = O

alcohols and **15** could be the C-20 epimer of **34**, would require the ketones **13** and **35** to be nearly identical.

(15) The exclusiveness of the loss of one bridge eliminates a stepwise process initiated by cleavage of the C-12–C-19 bond (which is both benzylic and α to nitrogen) because both bridges become equivalent if the resulting radical ion has sufficient lifetime for C-12 and C-19 to become planar. On the other hand, a stepwise mechanism involving initial cleavage at C-2–C-21 is not only consistent with all data but also accounts for the stereospecificity of the elimination of ethylene. This cleavage and the subsequent planar configuration of the bonds at C-2 release the considerable strain of the bridged ring system, whereas the alternative cleavage at C-2–C-3 not only leads to increased ring strain, but also greater steric crowding between C-4 and C-10.



(14) N. J. Leonard and R. C. Sentz, *J. Am. Chem. Soc.*, 74, 1704 (1952), and references therein.

(16) H. K. Schnoes and K. Biemann, *J. Am. Chem. Soc.*, **86**, 5693 (1964).

This was ruled out by a comparison of their mass spectra which were dissimilar far beyond the degree attributable to their difference in aromatic substitution [**13**: *m/e* 338 (M), 215 (a), 187, 169, 155, 124; **35**: *m/e* 294 (M), 266 (M − CO and M − C_2H_4), 252 (M − C_2H_2O), 138, 109[16]]. It follows that the ketone functions of **13** and **35** must be on different bridges, and the kosingine derivatives are therefore substituted at C-3 and/or C-4.

Kopsaporine and Kopsingarine. Kopsaporine, a second major alkaloid of *Kopsia singapurensis*, has a composition of $C_{23}H_{26}N_2O_6$, based on conventional analysis and mass spectrometric molecular weight. The mass spectrum suggested it to be demethoxykopsingine (**5**) because it was analogous to the spectrum of kopsingine except that all peaks above *m/e* 200 appeared 30 mass units lower. All other evidence agreed with this assignment. Analysis had indicated the presence of two methoxyl groups which, by analogy with kopsingine, could be attributed to a hydrogen-bonded urethan and an ester (γ_{max} 1675 and 1740 cm^{-1}; nmr 3.80 and 3.90 ppm). The nmr spectrum of kopsaporine showed the same olefinic absorption pattern [5.83 (J = 10 cps) and 6.02 ppm (J = 10, 4, and 2 cps)] as had been observed for kopsingine, and the ultraviolet spectrum [λ_{max}^{MeOH} 207 mμ (log ε 4.53), 246 (4.15), 279 (3.43), 287 (3.39)] was similar to the spectra of pleiocarpine (**1**)[3] and kopsine (**2**).[5] Three active hydrogens had been indicated by conventional analysis; however, only two deuterium atoms were incorporated by exchange with deuterium oxide in the mass spectrometer.

Further proof is the appearance of a fourth aromatic hydrogen in the nmr spectrum of kopsaporine, which was distinctive in its absorption as a broad doublet (7.53 ppm, J = 8 cps) slightly downfield from the remaining aromatic signals. This behavior is characteristic of the C-17 hydrogen atom of other N-carbomethoxyindoline alkaloids (*cf.* pleiocarpine[4]). Lack of this signal in the spectrum of kopsingine provides, in retrospect, complementary evidence for the assignment of a methoxyl group at C-17 in this alkaloid.

It is suggested that kopsingarine, also isolated from *K. singapurensis*, is 17-hydroxykopsaporine (**6**). This follows from a comparison of ultraviolet and infrared spectra of kopsingarine with data from kopsingine and kopsaporine.[2] Structure **6** is also in agreement with the elemental analysis of $C_{23}H_{28-30}N_2O_7$, including the presence of only two O-methyl groups. However, neither chemical nor mass spectral data have yet been obtained.

Experimental Section

Reaction products were usually purified by preparative thin layer chromatography (tlc) on silica gel H (Brinkmann), prewashed with methanol, using chloroform–methanol (9:1) as the solvent system. Compounds were detected with iodine vapor. The small scale on which reactions were frequently performed often precluded crystallization, and thus many compounds were characterized primarily by infrared, ultraviolet, and mass spectra.

Melting points were taken on a Kofler micro hot stage, and are uncorrected. Quantitative ultraviolet spectra were determined with a Cary Model 14 recording spectrophotometer, and those reported without extinction values were determined with a Cary Model 11 spectrophotometer. Infrared spectra were obtained using either a Perkin-Elmer Model 237B or Model 337 spectrophotometer. Nmr spectra were obtained in deuteriochloroform with a Varian A-60 spectrometer, and data are expressed in parts per million downfield from an internal tetramethylsilane standard.

Conventional mass spectra were determined with a CEC 21-103C mass spectrometer, equipped with a vacuum lock for the introduction of compounds of low volatility or thermal instability directly into the ion source. All spectra were recorded at 70 ev ionizing potential. High-resolution data were obtained with a CEC 21-110 double-focusing mass spectrometer, using a photographic plate for recording.

The spectral data reported here for kopsingine and kopsaporine were determined recently and some of them differ slightly from those previously reported,[2] which is due to the use of different instruments.

Isolation of Alkaloids. The isolation, reported in detail elsewhere,[2] involved alumina chromatography of the bases, obtained from a methanol extract of the leaves of *Kopsia singapurensis*. After separation of crystalline kopsingine from the early fractions, the remaining residue was subjected to countercurrent distribution giving kopsaporine, kopsingarine, and additional kopsingine.

Kopsingine (4). Recrystallization from chloroform–ethanol gave colorless prisms: mp 270–274° dec; pK_a = 5.27; $[\alpha]^{29}D$ +75°; λ_{max}^{MeOH} 217 mμ (log ε 4.56), 253 (4.04), 282 (3.38), 288 (3.36); $\nu_{max}^{CHCl_3}$ 1670, 1740, 3370 cm^{-1}; nmr 3.3 [multiplet, (C-8) H_2], 3.77 (singlet, OCH_3), 3.79 (singlet, OCH_3), 3.82 (singlet, OCH_3), 5.62 [doublet, J = 10 cps, (C-6) H], 5.73 [singlet, (C-3) OH], 5.95 [octet, J = 10, 4, and 2 cps, (C-7) H], 6.6–7.2 (multiplet, 3 H), 7.98 ppm [broad doublet, J = 4 cps, (C-4) OH]; mass spectrum, *m/e* 456 (M), 428, 427, 405, 397, 395, 379, 368, 339, 337, 283, 246.

Kopsaporine (5). Recrystallization from ethanol gave colorless needles: mp 234° dec; pK_a = 5.63; $[\alpha]^{27}D$ +48°; λ_{max}^{MeOH} 207 mμ (log ε 4.53), 246 (4.15), 279 (3.43), 287 (3.39); $\nu_{max}^{CHCl_3}$ 1675, 1740, 3350 cm^{-1}; nmr 3.4 [multiplet, (C-8) H_2], 3.80 (singlet, OCH_3), 3.90 (singlet, OCH_3), 5.83 [doublet, J = 10 cps, (C-6) H], 6.02 [octet, J = 10, 4, and 2 cps, (C-7) H], 6.32 [singlet, (C-3) OH], 7.0–7.4 (multiplet, 3 H), 7.53 [complex doublet, J = 8 cps, (C-17) H], 8.15 ppm [broad doublet, J = 5 cps, (C-4) OH]; mass spectrum, *m/e* 426 (M), 398, 397, 375, 367, 365, 349, 338, 309, 307, 253, 216, 156, 143, 130, 115, 108.

Kopsingine Acetate. Kopsingine (50 mg) and 50 μl of acetic anhydride were refluxed for 24 hr in 2 ml of pyridine. The product was evaporated to dryness under vacuum and the residue purified by tlc to give amorphous kopsingine acetate; $\nu_{max}^{CHCl_3}$ 1665, 1740, 3320 cm^{-1}; nmr 1.92 (singlet, CH_3CO), 3.67 (singlet, OCH_3), 3.77 (singlet, OCH_3) 3.80 (singlet, OCH_3), 5.15 [doublet, J = 2 cps, (C-4) H], 5.63 [(C-6) H], 5.78 [(C-7) H], 6.27 [singlet, (C-3) OH], 6.6–7.2 ppm (3 H); mass spectrum, *m/e* 498 (M), 479, 455, 439, 421, 379, 337 283, 123, 120, 107.

Dihydrokopsingine (7). Kopsingine (1.00 g), with 200 mg of platinum oxide in 50 ml of acetic acid–methanol (4:1), was reduced with hydrogen gas for 3 hr at room temperature and atmospheric pressure. After removal of platinum by filtration, the methanol was evaporated and 20 ml of water was added. The solution was made basic with sodium carbonate and then extracted with three 20-ml portions of chloroform. The extracts were combined, evaporated, and crystallized from chloroform–methanol to give 884 mg of dihydrokopsingine (**7**) as colorless prisms: mp 268–270° dec; λ_{max}^{MeOH} 216 mμ (log ε 4.58), 253 (4.06), 281 (3.38), 288 (3.36); $\nu_{max}^{CHCl_3}$ 1670, 1740, 3400 cm^{-1}; nmr 3.80 (singlet, 6 H, OCH_3), 3.82 (singlet, OCH_3), 5.62 [singlet, (C-3) OH], 6.6–7.2 (3 H), 8.44 ppm [doublet, J = 7 cps, (C-4) OH]; mass spectrum, *m/e* 458 (M), 443, 430, 429, 399, 370, 355, 341, 399, 315, 301, 278, 124, 122, 109.

Dihydrokopsingine Tosylate (9). Dihydrokopsingine (**7**, 50 mg) and 25 mg of *p*-toluenesulfonyl chloride were dissolved in 0.5 ml of pyridine and allowed to stand at room temperature for 24 hr. The solvent was removed under vacuum and the residue taken up in 5 ml of chloroform. This solution was rinsed successively with two 5-ml portions each of water, 0.5 *N* HCl, and saturated aqueous sodium carbonate, then dried over sodium sulfate and evaporated. The amorphous residue was not further purified and was identified as dihydroskopsingine tosylate on the basis of its nmr spectrum: 2.45 (tosylate CH_3), 3.45 (OCH_3), 3.74 (OCH_3), 3.80 (OCH_3), 4.90 [singlet, (C-4) H], 5.90 [singlet, (C-3) OH], 6.6–7.2 (3 H), 7.1–7.9 ppm (quartet, 4 H).

Hydride Reduction of Dihydrokopsingine to Triol 11. Dihydrokopsingine (**7**, 650 mg) and 350 mg of lithium aluminum hydride were refluxed for 4 hr in 50 ml of tetrahydrofuran. Excess hydride was decomposed with THF containing water, then solvents were removed by evaporation. Saturated sodium potassium tartrate solution (100 ml) was added, and the product was then extracted with three 50-ml portions of chloroform. After evaporation of the combined extracts, crystallization from ether yielded 375 mg of triol **11** as colorless needles. Recrystallization from ether gave:

mp 69–71°; λ_{max}^{MeOH} 216 mμ (log ε 4.40), 260 (3.87), 295 (3.34); $\nu_{max}^{CHCl_3}$ 3350, 3540 cm^{-1}; nmr 3.12 (singlet, NCH_3), 3.75 (singlet, OCH_3), 4.67 (doublet, J = 12 cps, *one* of the two hydroxymethylene hydrogens at C-3 with geminal coupling), 6.70 ppm (singlet, 3 H); mass spectrum, *m/e* 386 (M), 355, 327, 297, 271, 230, 216, 202, 188, 174, 154, 126, 125, 124, 112, 96.

Triol-(O-*d*)$_3$. When triol **11** was introduced into the mass spectrometer in the presence of deuterium oxide, the mass spectrum was that of triol-(O-*d*)$_3$ with peaks at *m/e* 389 (M), 357, 329, 298, 272, 231, 217, 203, 189, 174, 156, 128, 126, 124, 114, 97.

Triol-*d*$_5$ (12). Use of lithium aluminum deuteride in the reduction of dihydrokopsingine gave triol-*d*$_5$ (**12**): mass spectrum, *m/e* 391 (M), 358, 330, 300, 274, 235, 221, 205, 191, 177, 154, 126, 124, 112, 96.

Hydride Reduction of Dihydrokopsingine Tosylate to Diol 10. Dihydrokopsingine tosylate (approximate 50 mg) in 5 ml of tetrahydrofuran was refluxed for 2 hr with excess lithium aluminum hydride. The solvent was then evaporated and 50 ml of saturated sodium potassium tartrate solution was added. Two 10-ml extractions with chloroform, followed by tlc purification, gave 10 mg of diol **10** as the major product, with a smaller amount of triol **11**. The amorphous diol had $\nu_{max}^{CHCl_3}$ 3380 cm^{-1}; mass spectrum, *m/e* 370 (M), 355, 339, 311, 124, 122, 110, 96.

Periodate Cleavage of Diol 10 to Ketone 13. Diol **10** (9 mg) in 50 μl of methanol was added to 2 ml of 0.01% sulfuric acid–methanol which contained 40 mg of sodium periodate and 0.4 ml of water. The mixture was stirred at room temperature for 15 min and then poured into 10 ml of saturated sodium carbonate solution. Two extractions of 5 ml of chloroform each, followed by tlc purification, gave 2 mg of ketone **13**: $\gamma_{max}^{CHCl_3}$ 1720 cm^{-1}; mass spectrum, *m/e* 338 (M), 215 (a), 187, 155, 124.

Ketone-*d*$_2$ 14. Ketone **13** (2 mg) was dissolved in 1 ml of tetrahydrofuran. Deuterium oxide (0.1 ml) and a small piece of sodium metal were added, and the solution was stirred at room temperature for 4.5 hr. The sodium hydroxide layer was removed and 200 μl of deuterium oxide and additional sodium were added. After a total of 18 hr with stirring, the tetrahydrofuran was evaporated. Deuterium oxide (5 ml) was added and the product extracted with two 5-ml portions of chloroform to give ketone-*d*$_2$ **14**: mass spectrum, *m/e* 340 (M), 215, 187, 156, 126.

Deuteride Reduction of Ketone 13 to Alcohol 15. Ketone **13** (2 mg) was dissolved in 0.5 ml of tetrahydrofuran and excess lithium aluminum deuteride was added. After 3 hr at room temperature, the solvent was evaporated and 5 ml of saturated sodium potassium tartrate solution was added. Extraction with 5 ml of chloroform provided the pure deuterated alcohol **15**: mass spectrum, *m/e* 341 (M), 125, 110, 96.

Wolff–Kishner Reduction of Ketone 13 to 17-Methoxy-N-methylaspidofractinine (16). Ketone **13** (2 mg) was dissolved in 200 μl of ethanol. Hydrazine hydrate (20 μl) was added, and the solution was heated in a sealed tube for 5 hr at 100°. The ethanol was evaporated and 100 μl of 10% sodium hydroxide in ethanol was added. This mixture, sealed, was kept at 190° for 5.5 hr. Water (5 ml) was added, and the product was extracted with two 5-ml portions of chloroform to give pure 17-methoxy-N-methylaspidofractinine (**16**): λ_{max}^{MeOH} 218, 258, 293 mμ; mass spectrum, Figure 1.

Wolff–Kishner Reduction of Ketone 14 to 17-Methoxy-N-methylaspidofractinine-*d*$_4$ (17). Ketone **14** (2 mg) was dissolved in 100 μl of methanol-*d* and added to 100 μl of methanol-*d* containing 3 μl of anhydrous hydrazine. The solution was heated at 100° (sealed tube) for 2 hr and then the solvent was evaporated. A saturated solution (300 μl) of sodium methoxide in methanol-*d* was added, then sealed and heated at 200° for 4 hr. Water (5 ml) was added, and the product was extracted with two portions of 5 ml of chloroform. A mass spectrum indicated a small amount of deuterium in addition to that at C-3 and C-4, so the product was heated at 200° for 16 hr in 0.5 ml of sodium ethoxide–ethanol and worked up as above. After purification by tlc, the product had mass spectral peaks at *m/e* 328 (M), 300, 204, 190, 125, 111.

Periodate Cleavage of Triol 11. Triol **11** (300 mg) in 1 ml of methanol was added to 1.2 g of sodium periodate and 12 ml of water in 60 ml of 0.01% sulfuric acid–methanol. The mixture was stirred at room temperature for 15 min, then poured into 150 ml of saturated sodium carbonate solution. Four chloroform extractions of 25 ml each were combined and evaporated, giving 218 mg of amorphous residue. From 300 mg of this residue, after separation by tlc, three fractions were obtained containing diketone **27**, ketol **24**, and enediol **23**, each described below.

Diketone 27. Crystallization from methanol of the least polar fraction from the periodate cleavage of triol **11** gave 45 mg of diketone **27** as orange needles: mp 184–187°; λ_{max}^{MeOH} 216 mμ (log ε 4.48), 256 (3.92), 295 (3.45); $\nu_{max}^{CHCl_3}$ 1720, 1755 cm^{-1}; mass spectrum, *m/e* 352 (M), 296, 229, 226, 202, 109.

Ketol 24. The most polar of the three periodate cleavage fractions crystallized with difficulty from acetone to give 41 mg of ketol **24** as colorless prims: mp 190–193°; λ_{max}^{MeOH} 217 mμ (log ε 4.46), 257 (3.94), 296 (3.41); $\nu_{max}^{CHCl_3}$ 1715, 3350 cm^{-1}; nmr 3.02 (singlet, NCH_3), 3.42 [singlet, (C-19) H], 3.47 [(C-3) OH], 3.75 (singlet, OCH_3) 4.22 [singlet, (C-3) H], 6.6–7.1 ppm (3 H); mass spectrum, *m/e* 354 (M), 337, 326, 297, 203 (b), 124, 110, 96.

Ketol-*d* 25. Ketol **24** was dissolved in 1 ml of tetrahydrofuran with 100 μl of deuterium oxide. A small piece of sodium metal was added and the solution was allowed to stand at room temperature for 13 hr. The solvent was evaporated, 1 ml of deuterium oxide was added, and the product was extracted with 5 ml of chloroform. After purification by tlc, ketol-*d* **25** was obtained: mass spectrum, *m/e* 355 (M), 338, 327, 297, 204 (b′), 125, 110, 96.

Ketol-*d*$_2$ 26. When ketol-*d* **25** was introduced into the mass spectrometer in the presence of deuterium oxide, the following mass spectrum was obtained: *m/e* 356 (M), 338, 328, 298, 205 (b′), 125, 111, 97.

Enediol 23. The third fraction from the periodate cleavage of triol **11** could not be isolated or characterized in pure form. Even though chromatographic resolution was good, on repeated chromatography this fraction always contained large amounts of diketone **27** and ketol **24**, which must have formed by decomposition of the compound in question. The structure of an enediol **23** is postulated for this compound. An alternative ketol structure **22** cannot be ruled out, but it seems that the enediol would be more stable because of the trigonal carbon at C-4, and a stable 3,4 double bond has also been observed in the olefin **33**.

Diol 29. a. Diketone **27** (5 mg) with excess lithium aluminum hydride was allowed to stand at room temperature in 1 ml of tetrahydrofuran for 3 hr. The solvent was then evaporated and 5 ml of saturated sodium potassium tartrate was added. The product was extracted with two 5-ml portions of chloroform and crystallized from methanol to give colorless needles: mp 169–171°; $\nu_{max}^{CHCl_3}$ 3400 cm^{-1}; mass spectrum, *m/e* 356 (M), 297, 204 (c), 154, 126, 124, 112.

b. Ketol **24** (5 mg) was reduced with hydride in the same manner, giving a product identical with the diol **29** from the diketone (mmp 169–171°, identical infrared and mass spectra).

Diol-*d* 30. Ketol **24** was reduced as above, using lithium aluminum deuteride, giving diol-*d* **30**: mass spectrum, *m/e* 357 (M), 297, 204 (c), 155, 127, 124, 113.

Diol-*d*$_2$ 31. Diketone **27** was reduced as above, using lithium aluminum deuteride, giving diol-*d*$_2$ **31**: mass spectrum, *m/e* 358 (M), 297, 205 (c), 155, 127, 125, 113.

Wolff–Kishner Reduction of Diketone 27 to Alcohol 28. Diketone **27** (5 mg) and 5 μl of anhydrous hydrazine in 100 μl of ethanol was heated in a sealed tube at 100° for 3 hr. Evaporation under vacuum gave the monohydrazone: mol wt, 366; $\nu_{max}^{CHCl_3}$ 1665 cm^{-1} (chelated carbonyl). The hydrazone was dissolved in 100 μl of ethanol and 100 μl of a saturated solution of sodium ethoxide in ethanol was added. The reaction mixture was heated in a sealed tube at 200° for 4 hr. Water (5 ml) was then added, and the major product, alcohol **28**, was extracted with two 5-ml portions of chloroform, purified by tlc, and crystallized from methanol as colorless needles: mp 204–205°; mass spectrum, *m/e* 340 (M), 312, 188, 125, 124.

Wolff–Kishner Reduction of Ketol 24 to Alcohol 32 and Olefin 33. Ketone **24** (60 mg) in 0.5 ml of ethanol was heated at 100° in a sealed tube for 3 hr with 50 μl of anhydrous hydrazine. A saturated solution of sodium ethoxide in ethanol (0.5 ml) was added, and the mixture was then heated for 4 hr in a sealed tube at 200°. Water (25 ml) was added and the product was extracted with two 25-ml portions of chloroform. Separation by tlc provided two major products, alcohol **32** and olefin **33**.

a. Alcohol **32** (9 mg) was crystallized from methanol to give 5 mg of colorless prisms: mp 172–174°; λ_{max}^{MeOH} 217 mμ (log ε 4.41), 259 (3.85), 294 (3.37); mass spectrum, *m/e* 340 (M), 312, 188, 125, 124.

b. Olefin **33** (8 mg) was obtained in colorless amorphous form: λ_{max}^{MeOH} 257, 293 mμ; nmr 3.02 (singlet, NCH_3), 3.77 (singlet, OCH_3), 6.08 (doublet J = 9 cps), 6.45 (doublet, J = 9 cps), 6.6–7.1 ppm; mass spectrum, *m/e* 322 (M), 294 (d), 279, 266, 250, 238, 224, 161.

Reduction of Olefin 33 to 17-Methoxy-N-methylaspidofractinine (16). Olefin **33** (5 mg) in 1 ml of methanol was reduced with hydrogen over platinum at room temperature and pressure for 1 hr. Filtration and evaporation, followed by purification by tlc, yielded

a single amorphous compound which gave a mass spectrum identical with that of 17-methoxy-N-methylaspidofractinine (**16**), previously obtained from the ketone **13**.

Acknowledgment. This investigation was supported by a Public Health Service Fellowship (5-F1-GM-21,624-03) from the National Institute of General Medical Sciences (to D. W. T.) and a research grant of the National Science Foundation (GP 3734). The authors wish to thank Drs. B. Webster and C. Falshaw who performed some of the preliminary experiments.

Acceleration of Phenyl Ester Cleavage by Cycloamyloses. A Model for Enzymatic Specificity[1,2]

Robert L. VanEtten,[3] John F. Sebastian,[3] George A. Clowes, and Myron L. Bender

Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. *Received January 6, 1967*

Abstract: The cycloamyloses cause a markedly stereoselective acceleration of phenol release from a variety of substituted phenyl acetates in alkaline solution. Unlike methyl glucoside or glucose which produces small uniform rate effects, both cyclohexaamylose and cycloheptaamylose cause large, nonuniform increases in the rate of phenol release from *meta*-substituted phenyl acetates; phenol release from *para*-substituted phenyl acetates is only slightly enhanced. Rate effects due to cyclooctaamylose are smaller and much less stereoselective. The rate accelerations are independent of the electronic nature of the substituents. The cycloamylose system exhibits many characteristics of enzyme-catalyzed reactions, including saturation, competitive inhibition, and nonproductive binding. Dissociation constants of cycloamylose complexes with a variety of guest molecules were obtained using kinetic, spectroscopic, and competitive inhibition methods and are in experimental agreement. The maximal rate effects are independent of the stabilities of the cycloamylose–substrate complexes, as in enzymatic catalysis. The rate accelerations are entirely explained by considering the region of the cycloamylose torus and the secondary hydroxyl groups to be the active site of the cycloamylose. In the complex the *meta* substituents on the phenyl ring fix the carbonyl carbon atom of the ester in close proximity to the secondary hydroxyl groups of the cycloamylose whereas *para* substituents prevent this approximation. The reaction system constitutes a striking model for the lock and key theory of enzymatic specificity proposed by Emil Fischer.

As an aid to the understanding of the mechanism of enzyme action it would be useful to have a relatively simple model system which exhibits some of the important characteristics of enzyme-catalyzed reactions. Such a model system should exhibit substantial catalytic effects which vary in a predictable manner depending upon the substrate. The catalysis should be ascribable to a known reactive group or groups and the structure and geometry of the catalyst should be known. The catalytic effects should be the result of prior complexation of the catalyst and substrate. It must be possible to accurately determine both the stability of the catalyst–substrate complex and its inherent reactivity since there is no necessary relationship between these two factors (for example, although chymotrypsin binds N-acetyl-D-tryptophanamide somewhat better that it binds the L isomer,[4] the deacylation rates of N-acetyl-D- and -L-tryptophanyl chymotrypsins differ by a factor of 1.6×10^4 in favor of the L isomer[5]). Stereochemical aspects of both the binding and the subsequent catalysis should be readily explainable on the basis of the geometry of the catalyst and substrate. Other features of enzymic catalysis which should be sought for in a model system include competitive inhibition and nonproductive binding. In addition, it would be preferable if the catalysis proceeded by a mechanism similar to those so far known for enzyme-catalyzed reactions.

It appeared possible that a system incorporating the cycloamyloses[6-9] might permit the development of such a model. As the name implies,[10] the cycloamyloses are cyclic α-1,4-linked D-glucose polymers and have 6, 7, or 8 glucose residues per molecule.[11] X-Ray crystallographic studies have firmly established the structure[7]

(1) This research was supported by grants from the National Science Foundation.

(2) A preliminary report of this research has been published: M. L. Bender, R. L. VanEtten, G. A. Clowes, and J. F. Sebastian, *J. Am. Chem. Soc.*, **88**, 2318 (1966).

(3) National Institutes of Health Postdoctoral Research Fellow.

(4) The K_s for acetyl-L-tryptophanamide is 5.0×10^{-3} *M* [R. J. Foster and C. Niemann, *J. Am. Chem. Soc.*, 77, 1886 (1955)] while the K_i for acetyl-D-tryptophanamide is 2.3×10^{-3} *M* [R. J. Foster, H. J. Shine, and C. Niemann, *ibid.*, 77, 2378 (1955)].

(5) D. W. Ingles, J. R. Knowles, and J. A. Tomlinson, *Biochem. Biophys. Res. Commun.*, **23**, 619 (1966). Qualitatively similar observations may be made for K_s, K_i, and k_2 values of amide substrates but because reactions of the D isomers are so slow it is not possible to obtain an accurate ratio which describes the kinetic specificity of the k_2 step for chymotrypsin-catalyzed hydrolyses. Conversely, the k_2 step for chymotrypsin-catalyzed hydrolyses of esters related to specific substrates is too fast to permit a similar measurement.

(6) F. Cramer, "Einschlussverbindungen," Springer-Verlag, Heidelberg, 1954.

(7) D. French, *Advan. Carbohydrate Chem.*, 12, 189 (1957).

(8) J. A. Thoma and L. Stewart in "Starch: Chemistry and Technology," Vol. I, R. L. Whistler and E. F. Paschall, Ed., Academic Press Inc., New York, N. Y., 1965, p 209.

(9) F. R. Senti and S. Erlander in "Nonstoichiometric Compounds," L. Mandelcorn, Ed., Academic Press Inc., New York, N. Y., 1964, p 588.

(10) The names α-, β-, and γ-Schardinger dextrins and cyclodextrins became established early in the literature, the prefixes referring to cyclohexa-, -hepta-, and -octaamylose, respectively. Now that the structures of these molecules have been unequivocally established it seems appropriate to use the more descriptive amylose names.

(11) The structure of the homologous cyclononaamylose also appears to have been established: D. French, A. O. Pulley, J. A. Effenberger, M. A. Rougvie, and M. Abdullah, *Arch. Biochem., Biophys.*, **111**, 153 (1965).