4. *Alkaloids of* Kopsia *Species. Part I. Kopsine, Fruticosamine, and Fruticosine.*

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The alkaloids of *Kopsia fruticosa* are fractionated and three crystalline bases isolated. The formula of the previously known alkaloid kopsine is shown to be $C_{22}H_{24}N_2O_4$, and the two new alkaloids, fruticosamine and fruticosine, are isomeric with it. The chemistry of the three alkaloids is studied.

The genus *Kopsia* Bl. belongs to the subtribe *Vallesiinae* of the *Apocynaceae* family and comprises some thirty species of shrubs and small trees found in South-East Asia and Malaysia.[1] The presence of toxic alkaloids in *Kopsia arborea* Bl.* (*K. flavida*) has been long known [2-4] and one, kopsamine, was obtained crystalline [4] (cf. refs. 5 and 6). Later, three new bases were isolated [5,6] from the same plant (*K. longiflora*), were characterised, and were named kopsilongine, kopsiflorine, and kopsinine; of the four alkaloids, only the structure of kopsinine has been determined.[7]

Kopsia fruticosa (Ker) A. DC. yielded [2] an alkaloidal extract having a tetanus action, and a pure alkaloid, kopsine, isolated [8] from this source is claimed to be a hypotensive agent.[9] Kopsine was also isolated [10] in small amount from *K. arborea* (*K. albiflora*).

The chemistry of kopsine has been studied by Indian workers [8,11,12] who assigned the molecular formula $C_{22}H_{26}N_2O_4$ and proposed the structure (I) for this alkaloid. This

* The literature on *Kopsia* species is very confused. Many of the botanical names used are incorrect (ref. 1 and personal communication from Dr. N. G. Bisset), and we have adopted the system [1] of using *K. arborea* Bl. for the single plant material studied by various workers. The botanical names they used in their original papers are given in parentheses.

[1] Bisset, *Annales Bogorienses*, 1958, **3**, 105; 1961, **4**, 65.
[2] Greshoff, *Ber.*, 1890, **23**, 3537.
[3] van den Driessen Mareeuw, *Ned. Tijdschr. Pharm. Chem. Toxic.*, 1896, **8**, 199.
[4] Gorter, *Jaarb. Dep. Landb. Ned.-Ind.*, 1920, p. 240.
[5] Crow and Michael, *Austral. J. Chem.*, 1955, **8**, 129.
[6] Bisset, Crow, and Greet, *Austral. J. Chem.*, 1958, **11**, 388.
[7] Kump and Schmid, *Helv. Chim. Acta*, 1961, **44**, 1503; Battersby and Le Count, *J.*, 1962, 3245; Kump, Schmid, Le Count, and Battersby, *Helv. Chim. Acta*, 1962, **45**, 854.
[8] Bhattacharya, Chatterjee, and Bose, *J. Amer. Chem. Soc.*, 1949, **71**, 3370.
[9] Mukherjee, Maiti, and Dey, *J. Exp. Med. Sci.*, 1957, **1**, 179.
[10] Bhattacharya, *Science and Culture*, 1952, **18**, 293.
[11] Bhattacharya, *J. Amer. Chem. Soc.*, 1953, **75**, 381.
[12] Bhattacharya, *Science and Culture*, 1956, **22**, 120; Chatterjee, Abs. Hong Kong University Symposium on Natural Products, 1961.

structure is incompatible with the published ultraviolet and infrared spectra for kopsine and so need not be considered further; a re-investigation of kopsine and related alkaloids was therefore undertaken. The present paper reports isolation and study of the properties, functional groups, and relationships of the *Kopsia* alkaloids.

Alkaloids of *Kopsia fruticosa* (Ker) A. DC

	Kopsine (present work)	Kopsine (ref. 8 and 10)	Fruticosamine	Fruticosine
M. p.	210—214°	217—218° 216—217°	177—181°	225—226°
$[\alpha]_D^{20}$	−17·5° (*c* 2·15, $CHCl_3$)	+16·4° (EtOH)	+43° (*c* 2·73, $CHCl_3$)	−19° (*c* 2·60, $CHCl_3$)
pK_a' *	4·28	—	4·04	4·62
Ce^{4+} colour reaction on spot plate [13]	Purple → green → yellow	—	Transient purple → green → yellow	Intense blue-purple → green
R_K value †	1·0	—	1·3	0·85
Picrate	227—230° ‡	230° ‡	209—213° ‡	158—164° ‡
Methiodide	—	200° ‡	—	191—192° ‡

* In 4 : 1 2-methoxyethanol–H_2O. † Derived from thin-layer chromatograms run at 18—20° on silica gel G (Merck) in chloroform containing 5% methanol: R_K = (Distance moved by alkaloid)/(Distance moved by kopsine). ‡ With decomp.

The dried leaves of *K. fruticosa* used in the present work were collected in India (Calcutta), in Indonesia (Bogor), and in Malaya (Penang). These were extracted separately by percolation of the ground leaves with methanol, and the alkaloids were then isolated by an improved procedure (p. 29); all the samples were rich in bases, but contained little or no quaternary alkaloid. The Indian material readily yielded crystalline kopsine (0·85%) and further fractionation of the bases in the mother-liquors by countercurrent distribution gave a new alkaloid, m. p. 177—181° (0·1%), which we have named fruticosamine. No appreciable amount of kopsine could be found in the Malaysian and Indonesian leaves, the major alkaloid being fruticosamine (0·14—0·36%) which was separated by countercurrent distribution from a further new alkaloid, m. p. 225—226°, named fruticosine (0·05—0·12%). The properties of the three alkaloids are recorded in the annexed Table; a notable feature is the low basic strength of these alkaloids. The minor alkaloids present in various mother-liquors will be studied in future work.

The various plant specimens used above were carefully identified (see Acknowledgments), so their differing alkaloid contents may be the result of differing stages of development. Indeed, chromatographic examination of the alkaloids from a new batch of *K. fruticosa* collected near Calcutta suggests that the major alkaloids of this sample are fruticosamine and fruticosine (cf. sample above).

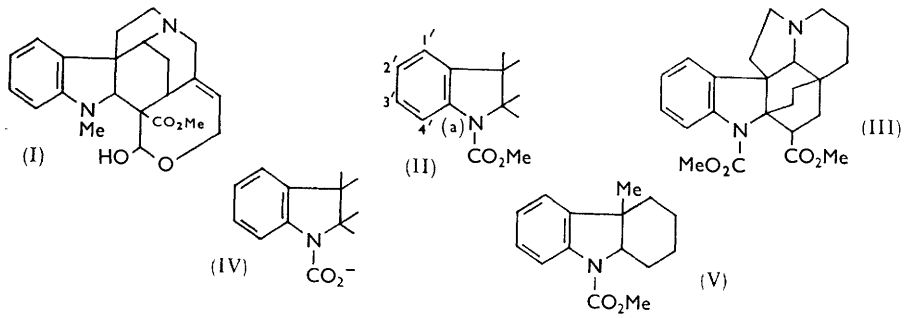
The work described in this paper was largely complete when a preliminary communication by Govindachari and his co-workers [14] became available to us. This outlines, without experimental detail, their work on kopsine alone.* On the basis of spectroscopic and chemical study they proposed the chromophore (II) for this alkaloid and recognised a cyclopentanone residue and a hydroxyl group; their other results are considered below. Our work on kopsine was carried out independently and, besides confirming many of the observations of the Madras workers, provides new evidence for these and other structural features.

Analyses of kopsine show that it contains one *O*-methyl group but no *N*-methyl or *C*-methyl group and that its molecular formula is $C_{22}H_{24}N_2O_4$, two hydrogen atoms fewer

* A second alkaloid (no molecular formula given) is reported to occur with kopsine which they name fruticosine. Only the m. p. is recorded and this (232°) is close to that of our fruticosine. The two bases could be identical since the second alkaloid in our leaves which contained kopsine was fruticosamine and this we have shown (below) to be converted very readily into fruticosine.

[13] Schmid, Kebrle, and Karrer, *Helv. Chim. Acta*, 1952, **35**, 1864.
[14] Govindachari, Rajappa, and Viswanathan, *J. Sci. Ind. Res. India*, 1961, **20**, *B*, 557.

than originally proposed;[8] the H_{24} formulation was rigorously confirmed by mass-spectrometric determination of molecular weight (found, 380). Titration of kopsine shows it to be a monoacid base. Its infrared spectrum (in $CHCl_3$) shows bands at 3315 (assignment as OH takes into account later evidence), 1750 (cyclopentanone), 1678 (>N·CO_2Me), and 750—770 (*o*-disubstituted benzene ring) cm.$^{-1}$, in Nujol. Only one band



is detectable in the –OH, –NH region and this, taken with the resistance of kopsine to acetic anhydride–pyridine under mild conditions is in keeping with the tertiary nature of N(b). Bhattacharya [11,12] had shown that kopsine is a derivative of indole and we confirm (cf. ref. 14) that the chromophore of kopsine is the system (II) recognised by its characteristic ultraviolet absorption [similar to that of pleiocarpine [7] (III)] which underwent a 5 mμ bathochromic shift when the solution was made 0·1N with respect to sodium hydroxide; this is interpreted as due to the generation of the anion (IV). Acidification of the alkaline solution caused a sharp drop in the intensity of absorption as would be expected since the *N*-carboxyindoline system will be decarboxylated and the generated basic centre will be protonated. The unusually ready hydrolysis of the urethane system in kopsine is demonstrated by the failure of the ultraviolet spectrum of the urethane [7] (V) to be affected by aqueous-ethanolic N-sodium hydroxide; the urethane had been dissolved in the alkali for 15 hr. at room temperature.

The nuclear magnetic resonance spectrum of kopsine * showed a sharp singlet [14] (3 protons) at 6·07 τ (>N·CO_2Me), and a singlet (1 proton) at 2·83 τ superimposed upon a multiplet (4 protons) extending over the region 2·2—3·0 τ. When the spectrum was redetermined after the solution of the kopsine had been shaken with deuterium oxide, the singlet at 2·83 τ had disappeared. This singlet is therefore assigned to the hydroxylic proton and the four-proton multiplet to the protons of the aromatic nucleus in partial structure (II). No signals corresponding to *C*-methyl, *N*-methyl, olefinic protons, or to a proton at position 2 of an acylindoline system [15,7] were present in the spectrum. Position 2 of structure (II) is therefore fully substituted. The absence of olefinic residues in kopsine (cf. ref. 14) is supported by perhydrogenation (below) and, on this basis, the molecular formula $C_{22}H_{24}N_2O_4$ requires there to be six rings in addition to the aromatic nucleus.

The foregoing information about kopsine can now be summarised in partial structure (VI); it is possible at this stage that the residue R may represent the hydroxyl group at present shown separately.

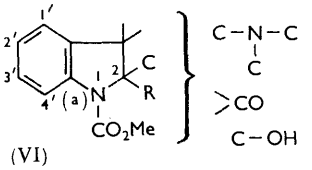
Reduction of kopsine with borohydride affected the ketonic carbonyl group and gave, in agreement with ref. 14, dihydrokopsine (VII), $C_{22}H_{26}N_2O_4$, which shows increased absorption in the infrared hydroxyl stretching region and loss of the 1750 cm.$^{-1}$ band. The urethane residue (II) was unaffected as shown by the ultraviolet spectrum of dihydrokopsine (as kopsine) and the 1675 cm.$^{-1}$ band (–N·CO_2Me) in its infrared spectrum, Dihydrokopsine formed a monomethiodide, $C_{23}H_{29}IN_2O_4$, which contained one *O*-methyl

* This and all other such spectra were measured in deuterochloroform at 60 Mc./sec.

[15] Djerassi, Archer, George, Gilbert, Schoolery, and Johnson, *Experientia*, 1960, **16**, 532.

and one *N*-methyl group; this salt still showed the ultraviolet spectrum of a methoxycarbonylindoline and thus the tertiary nature of N(b) in dihydrokopsine, and so in kopsine, is supported. Treatment of dihydrokopsine methiodide with cold alkali released no base extractable into 3 : 1 ether–chloroform.

Perhydrogenation of kopsine over platinum in aqueous sulphuric acid resulted in the uptake of 4·1 mol. of hydrogen. The crystalline hydrogenation product showed no selective



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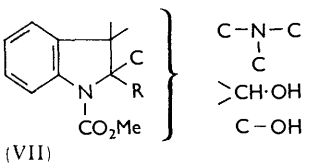

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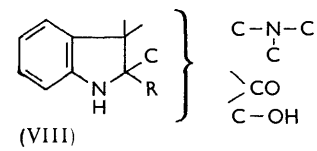

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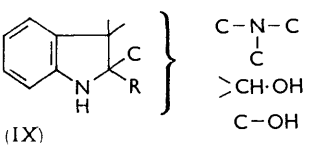


(IX)

absorption in the ultraviolet above 240 mμ, with rising end-absorption at shorter wavelengths. Also its infrared spectrum showed no bands in the 1700—1800 cm.$^{-1}$ region (cf. kopsine) and no bands corresponding to an aromatic nucleus. It follows that only the aromatic ring and the cyclopentanone have been reduced; this indicates that kopsine does not contain olefinic residues.

There had earlier been some confusion [8,11,12] about the alkaline hydrolysis of kopsine, but our results support and extend those of Govindachari *et al.* Whereas the alkaloid is affected only slowly by boiling 2N-hydrochloric acid, treatment of kopsine with 0·2N-sodium hydroxide for 30 min. at room temperature yields mainly demethoxycarbonylkopsine (VIII), $C_{20}H_{22}N_2O_2$ (indoline ultraviolet spectrum and colour reactions, $\nu_{max.}$ 1746 cm.$^{-1}$), formed as a result of the change $>N{\cdot}CO_2Me$ to $>NH$. By following the course of this reaction with the aid of thin-layer chromatography, we find that before all the kopsine has been hydrolysed, the demethoxycarbonylkopsine is being transformed into a new indoline base, m. p. 108—112°, which shows carbonyl absorption at 1757 cm.$^{-1}$ (in Nujol). Further studies are in progress on this product.

The ultraviolet spectra of demethoxycarbonylkopsine (VIII) and its dihydro-derivative (IX), prepared by borohydride reduction (cf. ref. 14), measured in aqueous-ethanolic N-hydrochloric acid both show considerable, but not complete, protonation at N(a); the N(a) nitrogen atom of these compounds is thus less basic than that of the Wieland–Gumlich aldehyde.[16] They are, however, stronger bases at N(a) than i strychanone (X), for the ultraviolet spectrum of this base is unaffected in 0·5N-methanolic hydrogen chloride.[17] In contrast, demethoxycarbonylkopsine is appreciably protonated at N(a) even in 0·1N-methanolic hydrogen chloride (fall to 1/5 of original ε-value) and this must be considered when siting the carbonyl group in proposals of structure. The spectrum of demethoxycarbonylkopsine measured in concentrated sulphuric acid shows almost complete protonation of N(a); the spectrum of a protonated indolenine (*e.g.*, XI) is not observed.

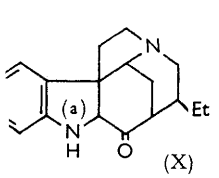
Permanganate oxidation of kopsine gave a mixture of neutral and acidic products. The acidic fraction was itself a mixture of at least four components (thin-layer chromatography), but the neutral fraction readily yielded oxokopsine,* a lactam, $C_{22}H_{22}N_2O_5$

* What is probably the same substance has been prepared by different methods [14] and was called lactam-A. Bhattacharya [12] reported that permanganate oxidation of kopsine gives oxokopsal, m. p. 280°, $C_{20}H_{22}N_2O_3$.

[16] Battersby and Hodson, *J.*, 1960, 786.

[17] Weissmann, Heshmat, Bernauer, Schmid, and Karrer, *Helv. Chim. Acta*, 1960, **43**, 1165.

(XII). This showed ultraviolet absorption very similar to that of kopsine and three carbonyl bands in the infrared spectrum (Nujol): 3300, 1770, 1690, and 1680 cm.$^{-1}$ (hydroxyl, cyclopentanone, $>N\cdot CO_2Me$, and six-membered lactam). It follows that N(b) of kopsine is contained in a six-membered ring with at least one methylene group adjacent to the



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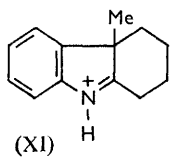

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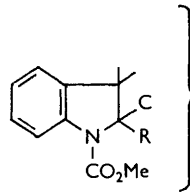
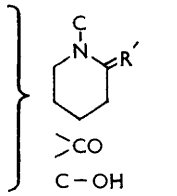


(XII; R = O)



(XIII; R = H_2)

nitrogen atom (XIII). This partial structure is not intended to exclude attachment of the hydroxyl group and/or the carbonyl group to the piperidine ring or to the carbon atom(s) attached to position 2 of the methoxycarbonylindoline system. The resistance of the hydroxyl group to oxidation supports the indication from attempted acetylation above that this group is tertiary.

The mass spectrum of kopsine is similar to those of fruticosine and fruticosamine (p. 28) and shows abundant ions at *M*/e 380, 352, 351, 282, 254, 223. No strong peaks appear at *M*/e 124, 110, or 109; comment is made on this aspect later. Further work is in progress aimed at the assignment of these ions and new chemical degradations are being studied (preliminary work shows that dihydrokopsine is cleaved by lead tetra-acetate).

Fruticosamine and fruticosine possess, like kopsine, the molecular formula $C_{22}H_{24}N_2O_4$. Many analyses on fruticosamine were low in carbon, apparently owing to tenacious retention of solvent. The molecular formula is established, however, by analysis of the monopicrate and monopicrolonate and by a mass-spectrometric molecular-weight determination on the alkaloid (found, 380). There were no analytical difficulties with fruticosine and its derivatives, and its molecular weight (mass spectrum) of 380 establishes the molecular formula.

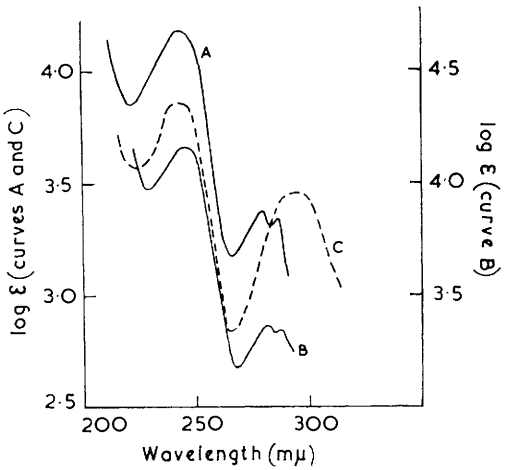
When fruticosamine is heated with 2N-hydrochloric acid, it is transformed into fruticosine and the same change is brought about readily by 0·2N-sodium hydroxide. The two related alkaloids can therefore be considered side by side. Titrations show them to be monoacid bases and they both contain one *O*-methyl group, but no *N*-methyl or *C*-methyl group. The ultraviolet spectra of the alkaloids (Figure) are similar to that of kopsine and are virtually unchanged by acid (neutral chromophore). They differ from kopsine in being less readily affected by alkali (cf. preparative work below). It was clear, however, that some hydrolysis had occurred since acidification of the solution caused a marked fall in the intensity of absorption (cf. kopsine above). The characteristic ultraviolet absorption of these alkaloids shows that they contain the methoxycarbonylindoline system (II); this chromophore is confirmed below.

The two alkaloids have similar infrared spectra (in $CHCl_3$) which show the following important bands: fruticosamine, 3410 (OH), 1730 (>CO), 1680 ($>N\cdot CO_2Me$), 750 (*o*-disubstituted benzene ring) cm.$^{-1}$, in Nujol; fruticosine, 3570 (OH), 1730 (>CO), 1683, ($>N\cdot CO_2Me$), 750—765 (*o*-disubstituted benzene ring) cm.$^{-1}$, in Nujol. Only the one band appeared in the OH, >NH region of the spectra.

The nuclear magnetic resonance spectra of the alkaloids give strong support to these results. Fruticosamine shows the following signals: a multiplet (4 protons of the aromatic ring) extending over the 2·3—3·2 τ region, a sharp singlet (3 protons) at 6·10 τ ($>N\cdot CO_2Me$) and a broad signal at 4·48 τ (1 proton) assigned to the hydroxylic proton since it was removed by the treatment with deuterium oxide used above for kopsine.

The spectrum of fruticosine shows several differences. The signal corresponding to the proton at position 4′ of partial structure (II) is separated from the rest and appears as a doublet with fine structure centred at 2·30 τ; the remaining protons of the aromatic ring show as a multiplet (3 protons) in the region 2·4—3·2 τ. A sharp singlet (3 protons appears at 6·12 τ (>N·CO_2Me) and a broader signal (1 proton) is centred at 6·83 τ; the latter was removed by treatment with deuterium oxide and is assigned to the hydroxylic proton. In addition to affecting the hydroxylic proton, this treatment changed a signal (1 proton), originally a quartet with fine structure centred at 5·21 τ, into a doublet (J = 6·5 c./sec.). This points to a system >CH—OH and further evidence is adduced below.

The spectra of fruticosamine and fruticosine both show signals close to, but not exactly in, the region (5·5—6·0 τ) where a proton at position 2 of an acylindoline absorbs.[7,15,18]



Ultraviolet absorption spectra of (A) fruticosamine in 1 : 1 aqueous ethanol (fruticosine shows an almost identical spectrum displaced 2 mμ to longer wavelengths), (B) fruticosamine or fruticosine in aqueous-ethanolic 0·1N-sodium hydroxide, and (C) demethoxycarbonylfruticosine in 1 : 1 aqueous ethanol.

Whether or not position 2 of these alkaloids is fully substituted must therefore be decided by future studies.

Mild acetylation of fruticosamine with pyridine–acetic anhydride at room temperature leaves it unchanged whereas these conditions convert fruticosine into its *O*-acetyl derivative, $C_{24}H_{26}N_2O_5$ [$\nu_{max.}$ (in $CHCl_3$), 1705 cm.$^{-1}$ and overlapping bands between 1720 and 1740 cm.$^{-1}$]. These results indicate that N(b) is tertiary in both alkaloids and, in the case of fruticosine, further support comes from methiodide formation (below).

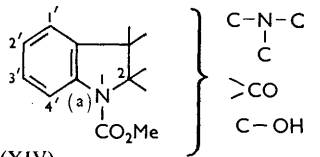
The nuclear magnetic resonance spectrum of *O*-acetylfruticosine supports the presence of the residue >CH·OH in the original alkaloid. Thus the quartet at 5·21 τ in the spectrum of fruticosine is replaced by a doublet (1 proton, J = 6·5 c./sec.) centred at 4·23 τ. This downfield shift of 0·98 p.p.m. is as expected for secondary alcohols.[19] Sharp singlets at 6·20 and 8·03 τ (3 protons each) correspond to >N·CO_2Me and O·COMe residues.

Fruticosamine is recovered unchanged after treatment with methyl iodide under conditions which cause partial conversion of fruticosine into its *N*(b)-methiodide $C_{23}H_{27}IN_2O_4$; this salt contains one *O*-methyl and one *N*-methyl group. Attempted reduction of the alkaloids with borohydride converted fruticosamine into fruticosine, owing to the basic medium, whereas fruticosine itself was unchanged under all conditions tested. Fruticosine also failed to form an oxime, so the carbonyl group must be strongly hindered.

The partial formula (XIV) can now be given to both alkaloids with the additional knowledge that for fruticosine, at least, the hydroxyl group is secondary.

[18] Djerassi, Brewer, Budzikiewicz, Orazi, and Corral, *Experientia*, 1962, **18**, 113.
[19] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 55.

Hydrolysis of fruticosine with sodium hydroxide was much slower than of kopsine and led to a mixture of two isomeric products, $C_{20}H_{22}N_2O_2$, named demethoxycarbonylfruticosine and isodemethoxycarbonylfruticosine. Boiling 2N-hydrochloric acid also effected this hydrolysis, but slowly, and *ca.* 50% of unchanged fruticosine was recovered after 6 hr. The two hydrolysis products were separated by countercurrent distribution but, because of their ready interconversion, even the best samples contained a trace of the isomer detectable by thin-layer chromatography. Starting with the two almost pure



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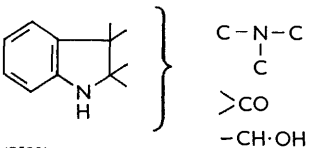

(XV)

isomers, each could readily be converted into a mixture of the two by acid or by base at room temperature. *O*-Methyl groups are absent from both products and the ultraviolet spectra correspond to the indoline chromophore. Both substances were considerably protonated on N(a) in aqueous-ethanolic N-hydrochloric acid and when demethoxycarbonylfruticosine was dissolved in concentrated sulphuric acid, the observed spectrum showed almost complete protonation of N(a). The protonated indolenine chromophore (XI) was not observed and knowing that the hydroxyl group is secondary, it is established that this group is not at position 2 of the indoline residue. The partial formula (XV) can now be given to the two hydrolysis products from fruticosine.

The low solubility of the two hydrolysis products prevented our examination of their infrared and nuclear magnetic resonance spectra under the conditions used above. However, the infrared spectra (in Nujol) of demethoxycarbonylfruticosine showed bands at 3480, 3420 (–OH, >NH) and 1708 cm.$^{-1}$ (>CO), and its isomer bands at 3350 (>NH), 1708 cm.$^{-1}$ (>CO) and a broad band 3200—2600 (strongly bonded OH) cm.$^{-1}$.

The removal of the methoxycarbonyl group from N(a) does not relieve the hindrance of the carbonyl group since the mixture of hydrolysis products from fruticosine was unaffected by borohydride. However, the mixture was converted by pyridine–acetic anhydride into one *NO*-diacetyl derivative, presumably owing to conversion of one of the isomers during the reaction. This product, $C_{24}H_{26}N_2O_4$, showed the ultraviolet spectrum of an *N*-acylindoline, which was unchanged by acid and alkali, and bands at 1746, 1728, and 1655 cm.$^{-1}$ in the infrared spectrum (in $CHCl_3$) corresponding to O·COMe, >CO, and >N·COMe. In the nuclear magnetic resonance spectrum, a doublet (1 proton, J = 6·5 c./sec.) again appeared at low field (4·07 τ) and sharp singlets (3 protons each) at 7·57 and 8·07 τ corresponding to >N·COMe and O·COMe.

The mass spectra of these alkaloids will be discussed in greater detail in a later paper. The main features are abundant ions for fruticosamine at M/e = 380, 352, 351, 282, and 243, and for fruticosine at M/e = 380, 352, 351, 321, 282, and 230. There are no major peaks in the spectra at M/e = 124, 110, or 109 which are characteristic ions from alkaloids of the pentacyclic and hexacyclic alkaloids of *Aspidosperma* and *Pleiocarpa* species.[7,20] However, skeletons of this general type must still be considered at this stage for the *Kopsia* alkaloids since a seventh ring could prevent the fragmentations which give rise to these ions. Further work aimed at elucidation of the structures of these alkaloids is in progress.

Recently Crow and Michael [21] have described further work on the alkaloids of *K. arborea* (*K. longiflora*), all of which differ from the alkaloids of *K. fruticosa*.

[20] Biemann, Friedmann-Spiteller, and Spiteller, *Tetrahedron Letters*, 1961, 485; Biemann and Spiteller, *ibid.*, 1961, 299; Gilbert, Ferreira, Öwellen, Swanholm, Budzikiewicz, Durham, and Djerassi, *ibid.*, 1962, 59; Djerassi, George, Finch, Lodish, Budzikiewicz, and Gilbert, *J. Amer. Chem. Soc.*, 1962, **88**, 1499.

[21] Crow and Michael, *Austral. J. Chem.*, 1962, **15**, 130.

Experimental

For general directions see Battersby, Binks, Hodson, and Yeowell.[22]

Isolation of Alkaloids.—(*a*) *Indian leaves.* Dried leaves of *K. fruticosa* (495 g.) were macerated with methanol in a Waring blender and then packed into a glass column. This was slowly percolated with methanol (12 l.), and the extract was evaporated to *ca.* 300 ml. After this had been diluted with acetic acid (75 ml.) and water (675 ml.), it was extracted with ether (5 × 500 ml.). The combined ethereal extracts were shaken with 2N-sulphuric acid (2 × 250 ml.), and this aqueous acid solution was basified with sodium carbonate before extraction again with ether (extract A).

The main aqueous acetic acid solution was adjusted to pH 10 with sodium carbonate and then extracted first with ether (6 × 750 ml.) to give extract B, and then with chloroform (3 × 750 ml.) to give extract C.

The final aqueous solution was adjusted to pH 2 with hydrochloric acid and treated with an excess of saturated aqueous ammonium reineckate. Only a trace of precipitate was formed.

Evaporation of extracts A and C left small amorphous residues which were examined further below. Extract B on evaporation left a residue which crystallised readily from methanol to yield kopsine (4·2 g.), m. p. 210—213°. This was shown to be homogeneous by countercurrent distribution (100 transfers) between 0·2M-sodium acetate–acetic acid buffer (pH 3·42) and 1 : 1 (v/v) ethyl acetate–light petroleum (b. p. 60—80°). Analysis showed one symmetrical peak, $K = 0·82$. The contents of the tubes corresponding to this peak were combined, the aqueous layer was adjusted to pH 9 with sodium carbonate, and the alkaloid extracted first with the organic layer and then with ethyl acetate. Evaporation of the combined organic extracts left kopsine which, recrystallised thrice from methanol, had m. p. 210—213° [Found: C, 69·2, 69·25; H, 6·35, 6·35; N, 7·2, 7·3; O, 17·0; OMe, 8·3; NMe, 0·0; *C*-Me, 0·0%; *M* (mass spectrum), 380. Calc. for $C_{22}H_{24}N_2O_4$: C, 69·45; H, 6·35; N, 7·35; O, 16·8; one OMe, 8·15%; *M*, 380], $\lambda_{max.}$ 241, 278, 285 mμ (log ε 4·09, 3·35, 3·33, respectively) in 1 : 1 aqueous ethanol.

The residue from evaporation of the mother-liquors from the crystallisation of kopsine was combined with the products from extracts A and C above and then fractionated by countercurrent distribution as in the foregoing experiment. This yielded, by the same working-up, kopsine (0·73 g.), m. p. 210—213°. The contents of tubes 70—100 were combined, basified and extracted as above, and the combined organic extracts were shaken twice with 2N-hydrochloric acid. After the combined acidic extracts had been basified with sodium carbonate, they were extracted thrice with ethyl acetate. Evaporation of the extracts left a residue which crystallised from methanol, to yield *fruticosamine* (0·4 g.), m. p. 177—181° [Found: C, 68·8, 68·3, 68·4, 68·2; H, 6·2, 6·5, 6·15, 6·3; N, 7·5, 7·65; O, 17·6; OMe, 8·55; NMe, 0·0; *C*-Me, 0·0%; *M* (mass spectrum), 380. $C_{22}H_{24}N_2O_4$ requires C, 69·45; H, 6·35; N, 7·35; O, 16·8; one OMe, 8·2%; *M*, 380. $C_{22}H_{24}N_2O_4,\frac{1}{2}$MeOH requires C, 68·2; H, 6·6; O, 18·15%), $\lambda_{max.}$ 243, 278, 286 mμ (log ε 4·17, 3·40, 3·37, respectively) in 1 : 1 aqueous ethanol.

The *picrate* of fruticosamine was prepared in ethanol as usual and, recrystallised from the same solvent, had m. p. 209—213° (decomp.) (Found: C, 54·9; H, 4·4; N, 11·2. $C_{28}H_{27}N_5O_{11}$ requires C, 55·2; H, 4·45; N, 11·5%).

A solution of fruticosamine (100 mg.) in ethanol was treated with picrolonic acid (75 mg.) and the precipitated salt was recrystallised several times from methanol, to give *fruticosamine picrolonate*, m. p. 278—280° (decomp.) (Found: C, 59·45; H, 5·05; N, 13·0. $C_{32}H_{32}N_6O_9$ requires C, 59·6; H, 5·0; N, 13·05%).

(*b*) *Malaysian and Indonesian leaves.* The Malaysian leaves (1·7 kg.) were extracted as under (*a*) but none of the three extracts A—C crystallised. They were therefore combined and fractionated by countercurrent distribution between 0·44M-sodium acetate–hydrochloric acid buffer of pH 1·42 and ethyl acetate for 100 transfers. Analysis showed two main peaks at K 0·11 and 0·66. The contents of the tubes corresponding to peak $K = 0·66$ were combined and worked up for alkaloid as above, to yield fruticosamine (2·32 g.), m. p. and mixed m. p. 177—181° (identical by infrared and thin-layer chromatography with above sample). The tubes corresponding to peak $K = 0·11$ similarly yielded *fruticosine* (0·91 g.), m. p. 225—226° after several recrystallisations from methanol and ethanol [Found: C, 69·3, 69·7; H, 6·2, 6·2;

[22] Battersby, Binks, Hodson, and Yeowell, *J.*, 1960, 1848.

N, 7·3, 7·35; O, 17·0; OMe, 8·55; NMe, 0·0; *C*-Me, 0·0%; *M* (mass spectrum), 380. $C_{22}H_{24}N_2O_4$ requires C, 69·45; H, 6·35; N, 7·35; O, 16·8; one OMe, 8·2%; *M*, 380], $\lambda_{max.}$ 245, 280, 286 mμ (log ε 4·18, 3·42, 3·38, respectively) in 1 : 1 aqueous ethanol.

The *picrate* of fruticosine was prepared as usual in ethanol and was recrystallised from benzene to give the benzene solvate, m. p. 158—164° (Found: C, 59·35; H, 4·9; N, 10·1. $C_{28}H_{27}N_5O_{11},C_6H_6$ requires C, 59·4; H, 4·8; N, 10·2%).

The Indonesian leaves (5 kg.) were extracted in the same way to give extracts A—C. The product from extract B gave, by countercurrent distribution as under (*b*), fruticosine (5·13 g.) and fruticosamine (3·5 g.), identified with above samples by m. p. and mixed m. p., infrared spectra, and thin-layer chromatography. In addition, extract A yielded a residue which crystallised directly from methanol to give fruticosamine (14·4 g.).

Dihydrokopsine.—Sodium borohydride (0·6 g.) was added portionwise in 1 hr. to a solution of kopsine (250 mg.) in methanol (25 ml.) at room temperature. The solution was kept for 24 hr. and the precipitated crystals were filtered off. After the filtrate had been diluted with dilute hydrochloric acid, it was extracted thrice with ethyl acetate, then basified with sodium carbonate and extracted again with ethyl acetate. Evaporation of the combined second set of extracts left a residue to which were added the above crystals; the whole recrystallised from methanol to yield *dihydrokopsine*, m. p. 250—252° (Found: C, 69·05; H, 6·9; N, 7·5. $C_{22}H_{26}N_2O_4$ requires C, 69·1; H, 6·85; N, 7·3%), $\lambda_{max.}$ 243, 278, 286 mμ (log ε 4·11, 3·36, 3·34, respectively) in 1 : 1 aqueous ethanol.

Sufficient methanol was added to a warm mixture of dihydrokopsine (31 mg.) and methyl iodide (2·5 ml.) to give a clear solution which was then heated under reflux for 4 hr. The residue obtained by evaporation was recrystallised four times from methanol–ethyl acetate to give *dihydrokopsine methiodide* (23 mg.), m. p. 235—238° (decomp.) (Found: C, 52·6; H, 5·9; N, 5·4; OMe, 5·95; NMe, 3·0. $C_{23}H_{29}IN_2O_4$ requires C, 52·6; H, 5·6; N, 5·35; one OMe, 5·9; one NMe, 2·9%), $\lambda_{max.}$ 225, 242·5, 278, 284 mμ (log ε 4·26, 4·15, 3·24, 3·21, respectively) in 1 : 1 aqueous ethanol.

Perhydrogenation of Kopsine.—A solution of kopsine (24·4 mg.) in 2N-sulphuric acid (10 ml.) was shaken with hydrogen and pre-reduced platinum (from 68·5 mg. of platinum oxide) at 20·5°/750 mm. (uptake 4·1 mol.). After filtration, the solution was extracted with ethyl acetate, then basified and extracted again with this solvent. Evaporation of the second set of extracts gave a residue which crystallised from ethanol to give the perhydrogenation product, m. p. 239—244°, $\varepsilon_{max.}$ (in $CHCl_3$) 3330, 1660 cm.$^{-1}$.

Demethoxycarbonylkopsine.—A solution of kopsine (382 mg.) in methanol (30 ml.) and 2N-aqueous sodium hydroxide (3 ml.) was kept at room temperature for 30 min., then diluted with water (60 ml.), and extracted thrice with ethyl acetate. Evaporation of the dried extracts gave a crystalline residue (124 mg.). The aqueous alkaline solution was acidified with hydrochloric acid, then basified with sodium carbonate and extracted thrice with ethyl acetate. These extracts on evaporation gave more (209 mg.) of the same material as obtained above (m. p. and mixed m. p., thin-layer chromatography). Recrystallisation of the combined material from methanol gave *demethoxycarbonylkopsine* (275 mg.), m. p. 232—234° (Found: C, 74·2; H, 6·95; N, 8·85; OMe, 0·0. $C_{20}H_{22}N_2O_2$ requires C, 74·5; H, 6·9; N, 8·7%), $\lambda_{max.}$ 239, 291 mμ (log ε 3·83, 3·48, respectively) in 1 : 1 aqueous ethanol. In 1 : 1 2N-aqueous hydrochloric acid–ethanol (*i.e.*, final solution N), the log ε values fell to 3·45 and 3·17, respectively, and a small peak appeared at 268 mμ; in 1 : 1 6N-aqueous hydrochloric acid–ethanol the original peaks had been almost entirely suppressed, to give the spectrum of a protonated indoline, $\lambda_{max.}$ 260 and 265 mμ (log ε ~2·75), and a very similar spectrum was observed when the solvent was concentrated sulphuric acid. Demethoxycarbonylkopsine in methanol showed $\lambda_{max.}$ 243, 297 mμ (log ε 3·86, 3·53, respectively) which were depressed to log ε 3·20, 2·08 when concentrated hydrochloric acid was added until the solution was 0·1N, and to log ε 2·92 and 2·66 when similarly adjusted to 0·5N.

Demethoxycarbonylkopsine gave an orange colour reaction with ceric sulphate.

The ultraviolet spectrum of 2,3,4,9-tetrahydro-11-methylcarbazole measured in concentrated sulphuric acid had $\lambda_{max.}$ 232 (shoulder), 235, 240, 283 mμ (log ε 3·73, 3·75, 3·73, 3·78, respectively).

Demethoxycarbonyldihydrokopsine.—A solution of demethoxycarbonylkopsine (60 mg.) in methanol (10 ml.) was treated portionwise with sodium borohydride (120 mg.) in 30 min. and then kept for 12 hr. at room temperature. The solution was acidified with hydrochloric acid,

then basified with sodium carbonate, which caused crystals to separate. These were collected (51 mg.) and a further quantity (9 mg.) of the same material was obtained by extraction of the filtrate with ethyl acetate. The total product was recrystallised several times from aqueous methanol to yield *demethoxycarbonyldihydrokopsine*, m. p. 262—264° (Found: C, 74·1; H, 7·45; N, 8·7. $C_{20}H_{24}N_2O_2$ requires C, 74·05; H, 7·45; N, 8·6%), $\lambda_{max.}$ 245, 296·5 mμ (log ε 3·82, 3·54, respectively) in 1 : 1 aqueous ethanol. In 1 : 1 2N-aqueous hydrochloric acid–ethanol the log ε at 245 mμ fell to 3·13.

Oxokopsine.—A solution of potassium permanganate (316 mg.) in stabilised acetone (50 ml.) was added dropwise to a boiling solution of kopsine (155 mg.) in dry acetone (10 ml.). After the mixture had been heated under reflux for 2 hr., it was cooled, the excess of oxidant was destroyed by formic acid, and the manganese dioxide was filtered off and washed with hot ethanol. The filtrate was treated with an excess of 2N-hydrochloric acid, freed from organic solvents by evaporation, and then extracted thrice with ethyl acetate. The combined ethyl acetate extracts were shaken with aqueous sodium carbonate, and the aqueous extracts were worked for acidic material [amorphous, 50 mg.; the derived methyl ester of this fraction (diazomethane) was shown to be a mixture on thin-layer chromatograms]. Evaporation of the ethyl acetate solution left the neutral fraction which was crystallised from methanol–di-isopropyl ether to afford *oxokopsine* (81 mg.), m. p. 225—227° (Found: C, 67·15; H, 5·55; N, 7·2. $C_{22}H_{22}N_2O_5$ requires C, 67·0; H, 5·6; N, 7·1%), $\lambda_{max.}$ 241, 277, 284 (log ε 4·05, 3·38, 3·37, respectively) in 1 : 1 aqueous ethanol.

Conversion of Fruticosamine into Fruticosine.—(*a*) *By acid*. A solution of fruticosamine (1·13 g.) in 2N-hydrochloric acid (18 ml.) was heated under reflux for 3 hr. and the cooled solution was then basified and extracted thrice with ethyl acetate. The dried extracts were evaporated and the residue was subjected to 30 transfers of countercurrent distribution in a 10-tube unit by the single-withdrawal technique in the system ethyl acetate–0·4M-aqueous sodium acetate–hydrochloric acid buffer at pH 2·6. The withdrawn ethyl acetate solution was evaporated, and the residue crystallised from methanol, to give fruticosine (723 mg.), m. p. and mixed m. p. 225—226°, further identified by infrared and thin-layer chromatography.

(*b*) *By base*. 2N-Sodium hydroxide (2·5 ml.) was added to fruticosamine (723 mg.) in methanol (25 ml.), and the solution was heated under reflux for 3 hr. After dilution with water, the solution was extracted thrice with ethyl acetate, and the extracts were evaporated to leave crystals (661 mg.), m. p. 218—222°, which were chromatographed over neutral alumina (activity I) in 1 : 1 chloroform–benzene and recrystallised several times from methanol, to yield pure fruticosine (207 mg.), identified by m. p. and mixed m. p. (225—227°), infrared spectrum, and thin-layer chromatography.

The aqueous alkaline solution was acidified with hydrochloric acid, basified with sodium carbonate, and extracted thrice with ethyl acetate. Evaporation of the extracts left deacylated material (80 mg.), m. p. 287—291°, which is discussed below.

Fruticosine Methiodide.—Fruticosine (170 mg.) was heated under reflux for 6 hr. with methyl iodide (10 ml.), the solution was evaporated, and the residue was crystallised from methanol–ethyl acetate, to give *fruticosine methiodide* (107 mg.), m. p. 191—192° (Found: C, 50·45; H, 5·15; N, 5·35; OMe, 6·2; NMe, 3·05. $C_{23}H_{27}IN_2O_4,H_2O$ requires C, 51·1; H, 5·4; N, 5·2; 1 OMe, 5·75; 1 NMe, 2·8%), $\lambda_{max.}$ 208, 224, 244, 284 mμ (log ε 4·53, 4·07, 3·97, 3·54, respectively) in 1 : 1 aqueous ethanol. The mother-liquors from this salt deposited crystals of unchanged fruticosine.

Fruticosine methiodide (20 mg.) was treated with sodium picrate (14 mg.) in aqueous ethanol; the precipitated *methopicrate*, when recrystallised from methanol, had m. p. 273—275° (Found: C, 55·95; H, 4·85; N, 11·1. $C_{29}H_{29}N_5O_{11}$ requires C, 55·9; H, 4·7; N, 11·25%).

O-*Acetylfruticosine*.—A solution of fruticosine (160 mg.) in pyridine (6 ml.) and acetic anhydride (2 ml.) was kept at room temperature for 24 hr. and then added dropwise with stirring into aqueous sodium carbonate solution at 0°. Extraction thrice with ethyl acetate yielded a gum which was converted into its picrate in ethanol (252 mg.), m. p. 165—168°. This was recrystallised several times from ethanol, and the final product run as a chloroform solution through a short column of neutral alumina (activity I). Evaporation of the percolate left O-*acetylfruticosine* which, recrystallised from light petroleum (b. p. 60—80°), had m. p. 115—118° (73 mg.) (Found: C, 68·55; H, 6·55; N, 6·3. $C_{24}H_{26}N_2O_5$ requires C, 68·25; H, 6·2; N, 6·6%), $\lambda_{max.}$ 243, 280, 287 mμ (log ε 4·13, 3·38, 3·35, respectively) in 1 : 1 aqueous ethanol.

Demethoxycarbonylfruticosine and Isodemethoxycarbonylfruticosine.—2N-Aqueous sodium

hydroxide (20 ml.) was added to a solution of fruticosine (689 mg.) in methanol (30 ml.) and after the solution had been kept at room temperature for 48 hr., with occasional warming to *ca.* 60°, it was diluted with water (100 ml.). Ethyl acetate extraction removed a crystalline product (247 mg.), m. p. 291—294°. The aqueous solution was acidified with hydrochloric acid, basified with sodium carbonate, and extracted thrice with ethyl acetate, to give more crystalline material (347 mg.), m. p. 290—294°. Thin-layer chromatography showed that both fractions contained the same two compounds. They were combined and added to the product from a similar hydrolysis of 1·09 g. of fruticosine, and the whole distributed for 175 transfers between ethyl acetate and 0·2M-sodium acetate–acetic acid buffer of pH 3·42. Analysis showed two peaks at $K = 0·30$ and 0·59 which were not completely separated; the tubes which contained both components were worked up to yield *ca.* 500 mg. of the mixture. The contents of the tubes corresponding to pure component with $K = 0·30$ were combined, and the aqueous layers basified with sodium carbonate and extracted with ethyl acetate to give *demethoxycarbonylfruticosine* (765 mg.) which, when crystallised several times from methanol had m. p. 292—295° (596 mg.) (Found: C, 74·4; H, 6·95; N, 8·5; OMe, 0·0. $C_{20}H_{22}N_2O_2$ requires C, 74·5; H, 6·9; N, 8·7%), $\lambda_{max.}$ 242, 294 mμ (log ε 3·85, 3·46, respectively) in 1 : 1 aqueous ethanol. In 1 : 1 2N-aqueous hydrochloric acid–ethanol these peaks had fallen to log ε 2·90 and 2·41, respectively, and the 267 mμ peak of the protonated indoline chromophore was visible. Further depression of the peaks occurred when the spectrum was measured in concentrated sulphuric acid.

A similar working-up of the tubes with pure component corresponding to $K = 0·59$ gave *isodemethoxycarbonylfruticosine* (198 mg.), m. p. 290—294° (Found: C, 74·15; H, 6·9; N, 8·65; OMe, 0·0. $C_{20}H_{22}N_2O_2$ requires C, 74·5; H, 6·9; N, 8·7%), $\lambda_{max.}$ 242, 293 mμ (log ε 3·86, 3·47, respectively) in 1 : 1 aqueous ethanol. In 1 : 1 2N-aqueous hydrochloric acid–ethanol, the peaks fell to log ε 2·96 and 2·57, respectively, and the 267 mμ peak was visible.

On thin-layer chromatograms of Merck silica gel G with 19 : 1 methanol–chloroform as the mobile phase, demethoxycarbonylfruticosine moved more rapidly than its isomer.

NO-*Diacetyldemethoxycarbonylfruticosine.*—The mixture of demethoxycarbonyl isomers (144 mg.) from hydrolysis of fruticosine was kept in solution with pyridine (6 ml.) and acetic anhydride (2 ml.) for 1 week. The solution was then added to aqueous sodium carbonate at 0° and extracted thrice with ethyl acetate, and the extracts were evaporated to dryness. A solution of the residue in ethanol was treated with a slight excess of picric acid, and the *picrate* of *NO*-diacetyldemethoxycarbonylfruticosine was recrystallised from ethanol (238 mg.); it had m. p. 244—247° (Found: C, 56·95; H, 4·8; N, 10·9. $C_{30}H_{29}N_5O_{11}$ requires C, 56·7; H, 4·6; N, 11·0%).

NO-*Diacetyldemethoxycarbonylfruticosine* was obtained by percolating a solution of the picrate (107 mg.) in chloroform over a column of neutral alumina (activity I). Evaporation of the percolate and crystallisation of the residue from di-isopropyl ether gave the pure derivative (46 mg.), m. p. 211—212° (Found: C, 70·9; H, 6·35; N, 7·65. $C_{24}H_{26}N_2O_4$ requires C, 70·9; H, 6·45; N, 6·9%), $\lambda_{max.}$ 209, 253, 280, 288 mμ (log ε 4·30, 4·12, 3·63, 3·54, respectively) in 1 : 1 aqueous ethanol.

[Added *November* 27th, 1962.] Recent developments in the chemistry of kopsine are (*a*) the suggestion that this alkaloid is related to the *Aspidosperma* alkaloids,[23] (*b*) the suggestion of several related structures for kopsine,[24] and (*c*) the suggestion of one structure for kopsine.[25]

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