1365. *Alkaloid Biosynthesis. Part IX.* The Ipecacuanha Alkaloids*

By A. R. Battersby, R. Binks, W. Lawrie, G. V. Parry, and B. R. Webster

The Ipecacuanha alkaloids have structures based upon the C_{9-10} unit (I) which is ubiquitous in the indole group of alkaloids. Four theories have been proposed to account for the biosynthesis of this unit. One makes use of an aromatic C_6–C_2 residue together with one or two one-carbon units. A second theory constructs the C_{9-10} system from three acetate units, a one-carbon unit, and a malonic acid residue. Strong evidence against both theories has been obtained by tracer experiments on the biosynthesis of cephaeline (II; R = H) in *Cephaelis ipecacuanha*; these experiments have further revealed the origin of the two isoquinoline systems in this alkaloid.

The third theory involves rearrangement of prephenic acid. This is discussed, using as a basis the results from tracer experiments which point against the involvement of a one-carbon unit in the genesis of the C_{9-10} system. The combined evidence indicates that further tracer studies are required to test rigorously the fourth published theory, based upon terpenoid precursors, and also the possibility that the C_{9-10} unit is derived from carbohydrate metabolism in a way not yet considered.

The structure and the illustrated absolute stereochemistry[1] of emetine (II; R = Me) have been established by many degradative studies[2] and the results have been confirmed by several syntheses.[3] Accompanying emetine in *Cephaelis ipecacuanha* is its phenolic relative, cephaeline (II; R = H), which can often be the major base present in the plants. These alkaloids and their close relatives are the only cases in the isoquinoline series to possess the C_{9-10} unit shown dissected in formula (I). This structural unit, as such or in apparently rearranged state, forms the reduced portion of the highly diverse structures found in the indole group of alkaloids. Corynantheine (III) serves as an example where the unit (I) can clearly be seen, and here the absolute stereochemistry corresponds to that of emetine.[4] In the Yohimbe, Aspidosperma, and Iboga alkaloids the C_{9-10} unit appears as (IV), (V), and (VI), respectively. The origin of this unit, is, at the time of writing, still one of the major unsolved problems in alkaloid biosynthesis, and several biogenetic schemes have been proposed to account for it; these will be considered below. Because of the structural relation of the Ipecacuanha and the indole alkaloids, biosynthetic information derived from the one assists research on the other. Both types of alkaloid have been studied by tracer methods in our Laboratory during the past five years and the present Paper reports the results derived for emetine and cephaeline. Preliminary accounts of part of this work have been published.[5,6]

* Part VIII, A. R. Battersby, D. M. Foulkes, and R. Binks, *J.*, 1965, 3323.

[1] A. R. Battersby and S. Garratt, *J.*, 1959, 3512; A. R. Battersby, R. Binks, and T. P. Edwards, *J.*, 1960, 3474; E. E. van Tamelen, P. E. Aldrich, and J. B. Hester, *J. Amer. Chem. Soc.*, 1959, **81**, 6214; Y. Ban, M. Terashima, and O. Yonemitsu, *Chem. and Ind.*, 1959, 568, 569; A. Brossi, A. Cohen, J. M. Osbond, P. Plattner, O. Schnider, and J. C. Wickens, *J.*, 1959, 3630.

[2] M. Pailer and K. Porschinski, *Monatsh.*, 1949, **80**, 94; A. R. Battersby and H. T. Openshaw, *J.*, 1949, 3207, and refs. therein.

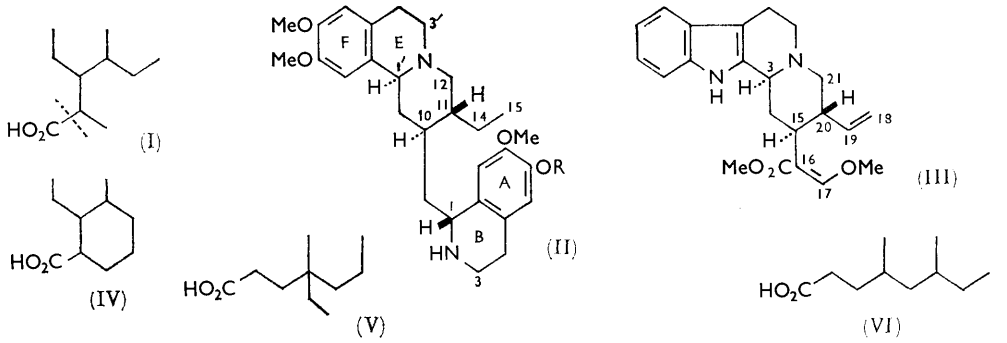
[3] *Inter alia*, (*a*) R. P. Evstigneeva and N. A. Preobrashenski, *Tetrahedron*, 1958, **4**, 223; (*b*) M. Barash, J. M. Osbond, and J. C. Wickens, *J.*, 1959, 3530; (*c*) A. Brossi, M. Baumann, and O. Schnider, *Helv. Chim. Acta*, 1959, **42**, 1515; (*d*) A. R. Battersby and J. C. Turner, *Chem. and Ind.*, 1958, 1324; *J.*, 1960, 717; (*e*) H. T. Openshaw and N. Whittaker, *J.*, 1963, 1461; (*f*) D. E. Clark, R. F. K. Meredith A. C. Ritchie, and T. Walker, *J.*, 1962, 2490.

[4] E. E. van Tamelen, P. E. Aldrich, and T. J. Katz, *J. Amer. Chem. Soc.*, 1957, **79**, 6426; A. R. Battersby and S. Garratt, *Proc. Chem. Soc.*, 1959, 86.

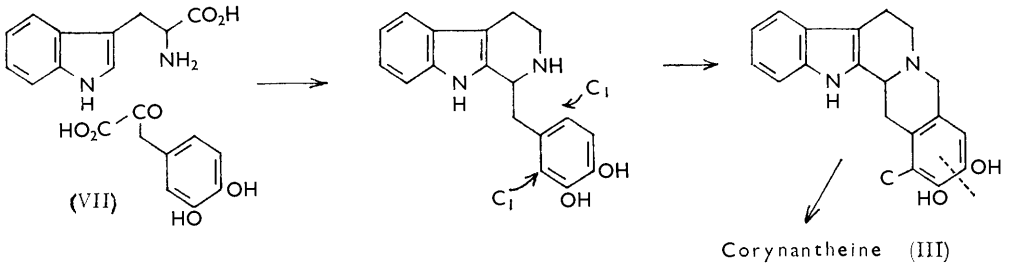
[5] A. R. Battersby, Donegani Lectures on Biosynthesis, Milan, Sept. 1962; Accad. Naz. dei Lincei, VII° Corso Estivo di Chimica, 1964, 47.

[6] A. R. Battersby, R. Binks, W. Lawrie, G. V. Parry, and B. R. Webster, *Proc. Chem. Soc.*, 1963, 369.

There are four biogenetic theories to consider. One is based upon " Woodward-fission " of a six-membered ring, initially proposed for the biosynthesis of strychnine [7] but often invoked since then for other indole alkaloids. This is shown for corynantheine (III) in Scheme 1, and, though the fission step is illustrated as occurring on a catechol



residue, reduction of the aromatic system could precede the ring-opening.[7] Robinson used the same approach for a postulated biosynthetic route to emetine; [8] this proposal is shown, slightly modified, in Scheme 2. Strong support for the last stage in Scheme 2 came from the isolation of the aldehyde (IX), named protoemetine, from *C. ipecacuanha*.[9]

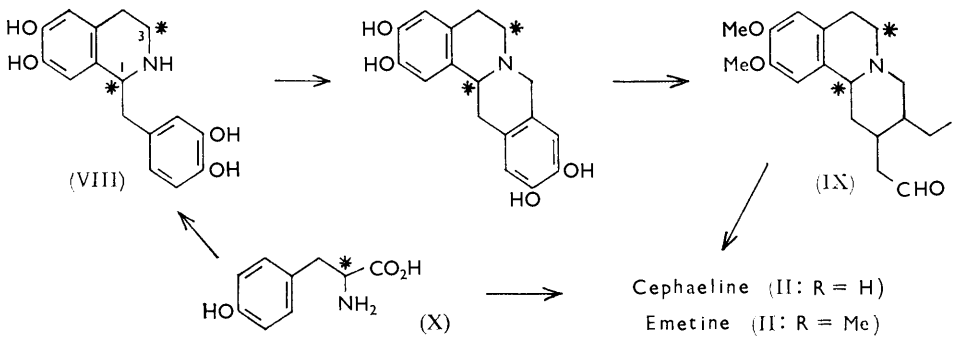


Scheme 1

The experimental test of this theory is difficult in the indole series since only one unit (*e.g.*, VII) derivable from tyrosine is involved; a negative result from incorporation experiments with labelled tyrosine can therefore be interpreted in ways other than that of eliminating the theory. This problem is overcome by working with the Ipecacuanha alkaloids, for, if Scheme 2 is correct, this would involve norlaudanosoline (VIII) or a close relative, which is known to be derived in higher plants from two C_6–C_2 units, in turn derivable from tyrosine.[10,11] Thus, [2-^{14}C]tyrosine (X) would lead on this basis to norlaudanosoline doubly-labelled at positions 1 and 3 which would finally yield cephaeline carrying three labels at positions 1′, 3′, and 3 [see (II; R = H)]. Whatever the origin of the C_{9-10} unit, it was *a priori* virtually certain that the phenethylamine systems of rings A,B and rings F,E (II) would be derivable from tyrosine [11] and would thus act as internal standards. A critical test of the theory that the C_{9-10} unit is based upon an aromatic C_6–C_2 unit and a one-carbon unit is thus available.

[7] R. B. Woodward, *Nature*, 1948, **162**, 155.
[8] R. Robinson, *Nature*, 1948, **162**, 524.
[9] A. R. Battersby, G. C. Davidson, and B. J. T. Harper, *J.*, 1959, 1744; A. R. Battersby and B. J. T. Harper, *J.*, 1959, 1748.
[10] *Inter alia*, A. R. Battersby and B. J. T. Harper, *J.*, 1962, 3526; A. R. Battersby, R. Binks, and B. J. T. Harper, *J.*, 1962, 3534; J. R. Gear and I. D. Spenser, *Canad. J. Chem.*, 1963, **41**, 783; A. R. Battersby and D. J. McCaldin, *Proc. Chem. Soc.*, 1962, 365; E. Leete, *J. Amer. Chem. Soc.*, 1959, **81**, 3948.
[11] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189.

Cephaelis ipecacuanha plants are difficult to cultivate, and artificial means were used to provide conditions on the Wirral peninsula similar to those of a South American rain forest. The radioactive cephaeline (II; R = H) from plants fed with (±)-[2-^{14}C]-tyrosine (Table 1) was methylated with diazomethane to afford emetine (II; R = Me) which was converted into its dimethiodide (XI); this is a crystalline solid, readily obtained



Scheme 2

in a solvent-free form, and its specific activity was taken in the early work as the standard for subsequent degradation products (Table 2). More recent studies employed emetine-*N*-phenylurea as the standard.[3c] The degradative methods used were those developed during the structural work on emetine [2] and these proven steps were followed to de-*N*(a)-tetrahydroemetinemethine (XII) and to de-*N*(a)-hexahydroemetinebismethine (XIV) Mild permanganate oxidation of these methines then gave two specimens of 6-ethylveratric acid corresponding to rings F and A, respectively. It was demonstrated that the dihydro-derivative of the methine (XII) did not give rise to 6-ethylveratric acid under these conditions. The activities of the two samples of 6-ethylveratric acid (Table 2) accounted for 95% of the original activity, and both acids afforded almost radio-inactive carbon dioxide on decarboxylation (Schmidt). Position 1′ of cephaeline (II; R = H) is thus proved not to be labelled, in contrast to the requirement of Scheme 2.

TABLE 1

Tracer experiments on *Cephaelis ipecacuanha*

Precursor	No. of plants	Year	Wt. of cephaeline (mg.)	Incorporation (%)
0·3 mc (±)-[2-^{14}C]Tyrosine	6	1960	300	0·8
0·1 mc (±)-[2-^{14}C]Phenylalanine	3	1961	41	0·018
0·5 mc Sodium [^{14}C]formate	4	1962	790	0·4
0·5 mc Sodium [1-^{14}C]acetate (Expt. 1)	3	1961	226	0·04
2·0 mc Sodium [1-^{14}C]acetate (Expt. 2)	12	1962	1560	0·04
0·33 mc Sodium [1-^{14}C]acetate (Expt. 3)	2	1963	82	0·04
0·33 mc Sodium [1-^{14}C]acetate + glucose (Expt. 4)	2	1963	72	0·04
0·33 mc Sodium [1-^{14}C]acetate + shikimic acid (Expt. 5)	2	1963	116	0·02
1·0 mc Sodium [1-^{14}C]acetate (Expt. 6)	4	1964	259	$1·4 \times 10^{-3}$
0·2 mc Sodium [1,3-^{14}C]malonate (Expt. A)	5	1963	484	0·06
0·25 mc Sodium [1,3-^{14}C]malonate (Expt. B)	4	1964	299	0·06

Kuhn–Roth oxidation of the 6-ethylveratric acid corresponding to ring A of cephaeline afforded acetic acid which was converted into methylamine by the Schmidt reaction. In this way (Table 2) it was proved that the original cephaeline (II; R = H) is labelled specifically at C-3. Insufficient material was available to allow the same approach to be used on the 6-ethylveratric acid corresponding to ring F. Accordingly, one portion of the radioactive emetine derived from the same batch of cephaeline was converted into the

N-phenylurea (II; R = Me, >NH = >N·CO·NHPh), and the rest by way of *N*-acetylemetine (II; R = Me; >NH = >NAc) into the methine (XV). Cleavage with osmium tetroxide–periodate [12] afforded formaldehyde having a specific activity (Table 2) which proves the original cephaeline to be specifically labelled at position 3′. No formaldehyde was produced when the dihydro-derivative of the base (XV) was subjected to the same conditions.

These results establish the biosynthetic origin of the two isoquinoline residues in cephaeline (II; R = H) and these internal standards make it certain that the C_9 unit of the Ipecacuanha alkaloids does not arise from tyrosine or from its biochemical relatives, such as *p*-hydroxyphenylpyruvic acid, 3,4-dihydroxyphenylalanine, or the corresponding keto-acid, in the way suggested in Scheme 2. This statement probably holds good also for the indole alkaloids because of the close structural relationship between the Ipecacuanha and the indole bases. Some support, though with the reservation noted above, comes from

TABLE 2

Degradation of radioactive cephaeline

Cephaeline and degradation products	Precursors and rel. activity [2-^{14}C]Tyrosine	
Emetine dimethiodide (XI)	1·00	
De-N(a)-Hexahydroemetinemethine methiodide (XIII)	1·00	
De-N(a)-Hexahydroemetinebismethine (XIV)	0·99	
6-Ethylveratric acid (ring A)	0·50	
6-Ethylveratric acid (ring F)	0·46	
Acetic acid, from ring A 6-ethylveratric acid	0·46	
Methylamine (Schmidt on acetic acid)	0·46	
Barium carbonate from ring A acid	<0·01	
Barium carbonate from ring F acid	<0·01	
Emetine *N*-phenylurea	1·00	
Formaldehyde dimethone (C-3′)	0·45	
	[2-^{14}C]Phenylalanine	Sodium [1-^{14}C]acetate (Expt. 2)
Cephaeline oxalate	1·00	1·00
N-Acetyldihydroemetinemethine methiodide	—	0·99
Amino-aldehyde (XIX)	[0·61]	[0·56]
6-Ethylveratric acid (ring F)	0·28	0·39
Barium carbonate from ring F acid	0·03	0·04
	Sodium [^{14}C]formate	
Cephaeline	1·00	
Triethylamine methiodide (Zeisel) × 3 *	0·67	
Emetine *N*-phenylurea	1·00	
N-Acetylemetine methiodide	0·97	
N-Acetyldihydroemetinemethine	0·98	
The ketone (XXVI)	0·99	
Formaldehyde dimethone	0·013	
p-Bromophenacyl acetate	0·022	
p-Bromophenacyl propionate	0·038	

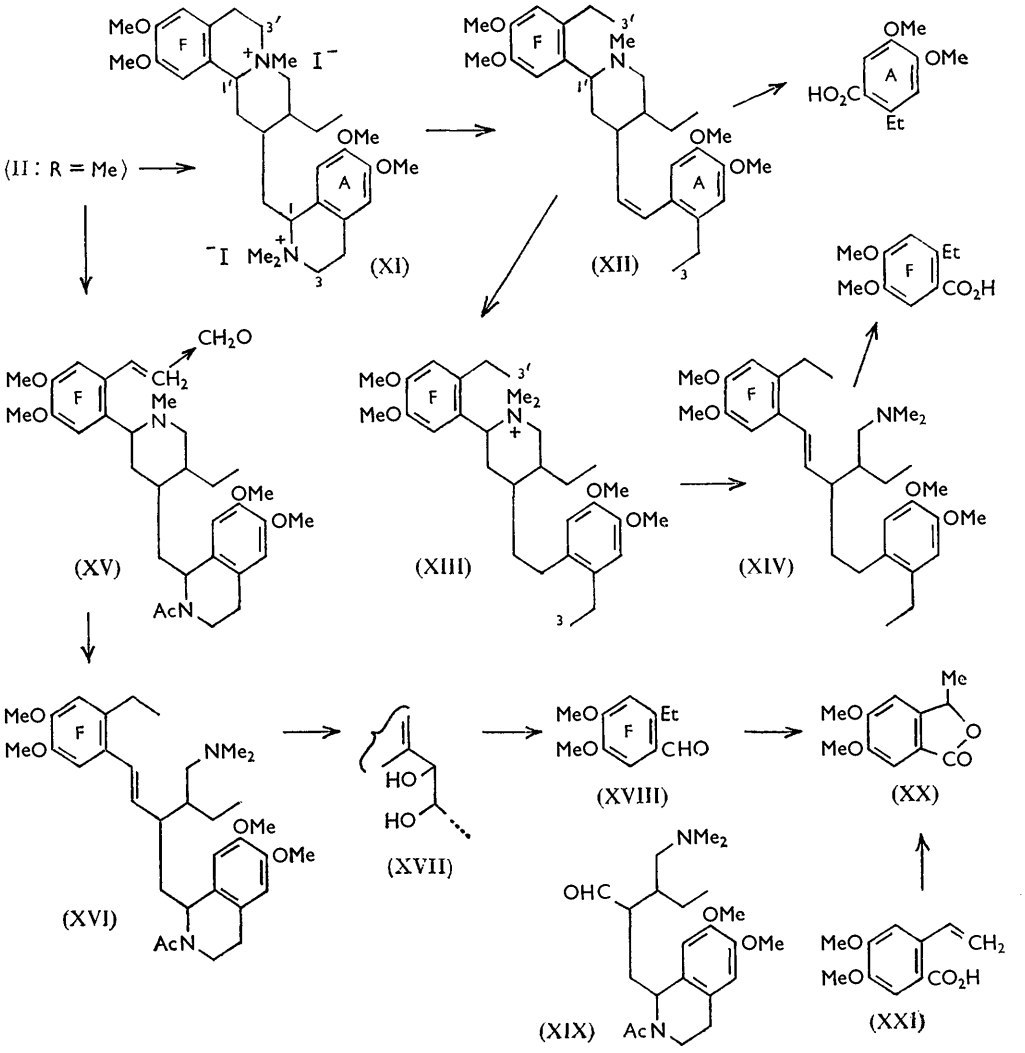
* It is necessary to multiply the relative molar activity of the methiodide by 3 in order to obtain the total relative activity in the *O*-methyl groups of cephaeline.

the failure to achieve incorporation of activity from labelled tyrosine into ajmaline (XXII) in *Rauwolfia serpentina*.[13] Further evidence against Scheme 2 is adduced below.

Evidence has been accumulating during the last few years that for many higher plants, *e.g.*, those of the *Amaryllidaceae* [14] and of *Colchicum*,[15] tyrosine and phenylalanine are not interconvertible. It follows that the foregoing experiments do not eliminate phenylalanine

[12] R. Pappo, D. S. Allen, jun., R. U. Lemieux, and W. S. Johnson, *J. Org. Chem.*, 1956, **21**, 478.
[13] E. Leete, S. Ghosal, and P. N. Edwards, *J. Amer. Chem. Soc.*, 1962, **84**, 1068.
[14] (*a*) R. J. Suhadolnik, A. G. Fischer, and J. Zulalian, *J. Amer. Chem. Soc.*, 1962, **84**, 4348; (*b*) W. C. Wildman, A. R. Battersby, and S. W. Breuer, *ibid.*, p. 4599; (*c*) A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J.*, 1964, 1595.
[15] (*a*) A. R. Battersby and J. J. Reynolds, *Proc. Chem. Soc.*, 1960, 346; (*b*) A. R. Battersby, R. Binks, J. J. Reynolds, and D. A. Yeowell, *J.*, 1964, 4257, and refs. therein; (*c*) E. Leete and P. E. Nemeth, *J. Amer. Chem. Soc.*, 1960, **82**, 6055; (*d*) E. Leete, *Tetrahedron Letters*, 1965, No. 5, 333.

or its biochemical equivalents as possible sources of the C_{9-10} unit. When (±)-[2^{14}-C]-phenylalanine was administered to the plants by wick, as for tyrosine above, there resulted only a low incorporation into cephaeline (Table 1), immediately pointing against this amino-acid as a true precursor. The derived emetine was degraded by way of the methine (XV) to *N*-acetyldihydroemetinebismethine (XVI). Hydroxylation of the olefinic residue with osmium tetroxide and cleavage of the resultant diol (XVII) with lead tetra-acetate yielded 6-ethylveratraldehyde (XVIII) and an amorphous product which was homogeneous by thin-layer chromatography; the latter substance is presumed to be the amino-aldehyde

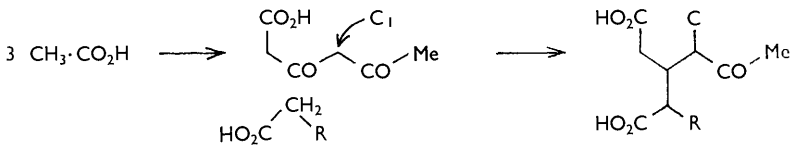


(XIX). The activity of this product was determined, but the value is set in parentheses (Table 2) since all other values reported are derived from analytically pure materials. Permanganate oxidation of the aldehyde (XVIII) led mainly to the lactone [16] (XX) but oxidation by alkaline hydrogen peroxide [17] afforded 6-ethylveratric acid which was decarboxylated as before. No significant activity was found in the carbon dioxide, which corresponds to C-1′ of cephaeline (II; R = H).

[16] E. Maekawa and Y. Sumimoto, *Bull. Chem. Soc. Japan*, 1960, **33**, 941.
[17] A. Dobrowsky, *Monatsh.*, 1955, **86**, 325.

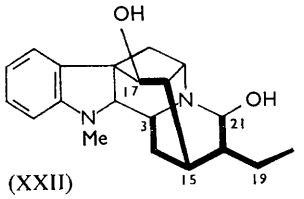
The lactone (XX) was prepared for comparison purposes by acid-catalysed cyclisation of 6-vinylveratric acid [18] (XXI).

It is convenient to consider next the biogenetic proposal based upon acetic and malonic acids [13,19] which, though chronologically third, has been extensively studied experimentally. Here the C_{9-10} unit is considered to be constructed from three acetate units, or their equivalents, a one-carbon unit, and a C_3 unit, which could be malonic acid (R = CO_2H) or formylacetic acid (R = CHO). In its essentials, this is illustrated in Scheme 3 which indicates how the unit (I) might be constructed. However, by linking the units in different ways, the systems (IV), (V), and (VI) can be accommodated. On this basis, [1-^{14}C]acetic acid should afford cephaeline labelled at positions 1', 10, and 14, and probably also at position 1. In the indole series, ajmaline (XXII) would be expected to become labelled at the corresponding positions 3, 15, and 19, and probably at 17, from the same precursor. The C_9 unit of ajmaline is shown with thickened bonds.

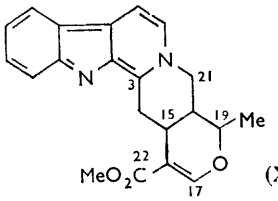


Scheme 3

Experimental support for this theory was provided in three Papers by Leete and his colleagues. The first reported [20] that C-21 of ajmaline (XXII) becomes labelled (12% of total activity) when sodium [^{14}C]formate is fed to *R. serpentina* plants, in agreement with its derivation from the one-carbon pool of the plants. The second [13] described the incorporation of sodium [1-^{14}C]acetate into ajmaline in exact agreement with theory; positions 3 and 19 each carried 26% of the total activity. Further support came from the reported [21] incorporation of sodium [1-^{14}C]acetate into serpentine (XXIII) to label C-19 with 23% of the total activity; C-22 was reported to be radio-inactive. The third Paper [21] further reported that [1,3-^{14}C]malonic acid was incorporated into serpentine (XXIII) having 48% of the total activity at position 22, and also into ajmaline such that 74% of the total activity appeared at C-17.



(XXII)



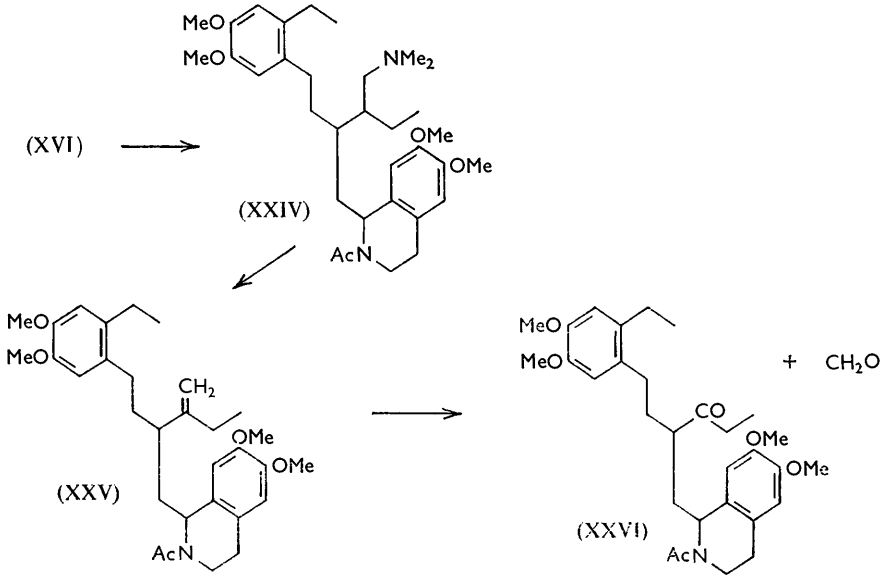
(XXIII)

All our results [6] on the biosynthesis of ajmaline in *R. serpentina* differ from those of Leete *et al.*; they will be described in full together with more recent findings in our complete Paper on the indole alkaloids. The work on the Ipecacuanha alkaloids described below agrees with our findings on ajmaline and is in sharp contrast to that summarised in the previous paragraph.

Sodium [^{14}C]formate was incorporated by *C. ipecacuanha* plants into cephaeline (Table 1), part of which was demethylated (Zeisel) to establish that 67% of the total activity was present in the *O*-methyl groups. This proved that the one-carbon pool of the plants had become strongly labelled. Kuhn–Roth oxidation of the emetine-*N*-phenylurea derived

[18] T. Kondo and N. Mori, *J. Pharm. Soc. Japan*, 1931, **51**, 615.
[19] E. Schlittler and W. I. Taylor, *Experientia*, 1960, **16**, 244; A. R. Battersby, quoted in footnote 11, ref. 13.
[20] P. N. Edwards and E. Leete, *Chem. and Ind.*, 1961, 1666.
[21] E. Leete and S. Ghosal, *Tetrahedron Letters*, 1962, 1179.

from a further part afforded acetic and propionic acids which were separated chromatographically. Their activities (Table 2) show low scatter of activity over the positions **11**, **14**, and **15** of cephaeline (II; R = H) at about the random skeletal level after allowance for the activity present in the *O*-methyl groups. The methods used to ensure experimental reliability of the separation procedures, the Kuhn–Roth and the Schmidt reactions are described in the Experimental section.



The remaining cephaeline was degraded by the route outlined above to the bismethine (XVI). Hydrogenation gave the tetrahydrobismethine (XXIV) which by Hofmann degradation afforded the olefin (XXV) and recovered base (XXIV); re-treatment of the latter led to an acceptable yield of the required material (XXV). This was cleaved by osmium tetroxide and periodate to yield formaldehyde and the crystalline ketone (XXVI); the dihydro-derivative of the olefin (XXV) was unaffected by these reagents. The interlocking evidence from the activities of these two products (Table 2) proves that position 12 of cephaeline, corresponding to position 21 of ajmaline, carries exactly the random level of activity. This is strong evidence against the involvement of a one-carbon unit at this position for the construction of the C_{9-10} unit, and our work on ajmaline [6] is in agreement.*

Sodium [1-^{14}C]acetate was incorporated in low yield into cephaeline (II; R = H) which was degraded as before by the Kuhn–Roth and Schmidt methods. Experiments 2 and 3 (Tables 2 and 3) showed that activity is spread along positions **11**, **14**, and **15** of

TABLE 3

Kuhn–Roth oxidation of radioactive cephaeline

	Total activity (%) in			
Precursor	$MeCO_2H$	$MeNH_2$	$EtCO_2H$	3 × OMe
Sodium [1-^{14}C]acetate (Expt. 2)	6·2	2·7	9·7	19
Sodium [1-^{14}C]acetate (Expt. 3)	6·0	—	9·3	—
Sodium [1-^{14}C]acetate (Expt. 4)	6·5	—	9·2	—
Sodium [1-^{14}C]acetate (Expt. 5)	6·9	—	9·8	—
Sodium [1-^{14}C]acetate (Expt. 6)	3·2	1·8	4·1	—
Sodium [1,3-^{14}C]malonate (Expt. B)	6·2	—	8·5	—

* We are grateful for personal communications from Professor D. Arigoni and Professor D. H. R. Barton with Dr. G. W. Kirby to the effect that the results from their separate studies in the indole series provide further evidence against the use of a one-carbon unit.

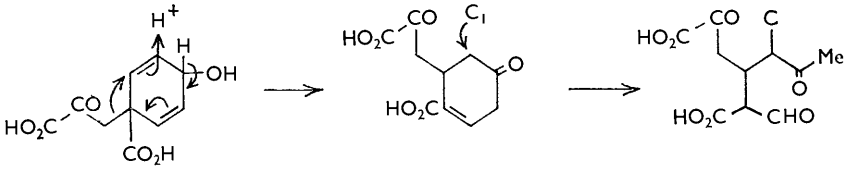
cephaeline and, importantly, that no high level of labelling appears at position 14 as would be required by the theory under test. Position 1′ of cephaeline was examined by degradation of the alkaloid from Expt. 2 (Tables 1 and 2) by the sequence (II; R = H) ⟶ (II; R = Me) ⟶ (XV) ⟶ (XVI) ⟶ (XVII) ⟶ 6-ethylveratric acid; the carboxyl group from the final product was isolated as carbon dioxide (Schmidt). It was thus shown that, whereas 6-ethylveratric acid contains over one third of the total activity (Table 2) of cephaeline, only the random level is located at the carboxyl group which corresponds to the original C-1′. On the acetate–malonate theory, this should be strongly labelled (Scheme 3). The far reaching extent to which scatter of activity has occurred is further shown (Table 3) by the appreciable labelling at the *O*-methyl groups, which are drawn from the one-carbon pool.

The labelling pattern of the 6-ethylveratric acid from Expt. 2 shows that those parts of cephaeline which we have proved to be derived from tyrosine, and thus to be on the carbohydrate–shikimic acid–prephenic acid pathway, have become labelled when sodium [1-^{14}C]acetate is the precursor. Expts. 4 and 5 were therefore carried out (Tables 1 and 3) in which inactive glucose and shikimic acid were administered at the same time as the labelled sodium acetate with the aim of reducing scatter. In the event, very similar results to those from Expts. 2 and 3 were obtained. Finally, the labelled sodium acetate was fed to plants kept throughout in darkness (Expt. 6); there resulted a reduced incorporation (Table 1) and again only low scatter of activity was present over positions 11, 14, and 15 (Table 3). In these recent experiments, the *O*-methylation of cephaeline to emetine was found to be more satisfactorily carried out by the trimethylphenylammonium hydroxide method.[22]

Similar scatter occurred when sodium [1,3-^{14}C]malonate was used as the precursor, as shown by the activities of the degradation products (Table 3). Clearly, extensive decarboxylation has taken place to give a labelling pattern over the *C*-ethyl group similar to that found consistently from sodium [1-^{14}C]acetate.

The foregoing results constitute powerful evidence against the acetate–malonate theory (Scheme 3), and we consider this theory to be incorrect.* Further, the demonstration that a one-carbon unit is not involved in the biosynthesis of the C_{9-10} unit adds further weight to the evidence presented earlier against Scheme 2.

The third theory [23] also requires a one-carbon unit to combine with the product formed by rearrangement of prephenic acid, as illustrated in Scheme 4. At present, the results



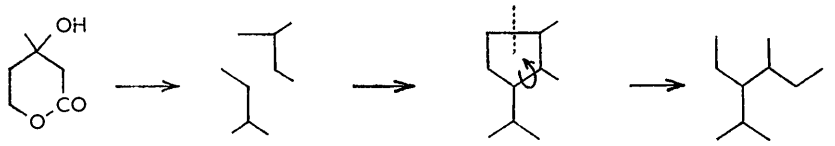
Scheme 4

concerning the one-carbon unit form the sole evidence against this proposal and further work is required here.

The only published theory which does not involve a C_1 residue for the construction of the C_{9-10} unit is the monoterpene theory [23,24] (Scheme 5). Our early experiments with (±) — [2-^{14}C]mevalonolactone on *R. serpentina* and *C. ipecacuanha* led, respectively, to

* Professor E. Leete has kindly informed us that he now holds this view.

[22] V. M. Rodinov, *Bull. Soc. chim. France*, 1926, [4], **39**, 305.
[23] E. Wenkert and N. V. Bringi, *J. Amer. Chem. Soc.*, 1959, **81**, 1474; E. Wenkert, *ibid.*, 1962, **84**, 98.
[24] R. Thomas, *Tetrahedron Letters*, 1961, 544.



Scheme 5

radio-inactive ajmaline (XXII) and cephaeline (II; R = H) but to a satisfactory and specific incorporation into β-sitosterol in the former plant.[25] Leete and his co-workers [13] also obtained negative results from this precursor for ajmaline. Our more recent experiments have afforded low incorporations of activity from sodium (±)-[2-^{14}C]mevalonate into cephaeline and ajmaline. Professor D. Arigoni and Professor A. I. Scott have obtained similar small incorporations in the indole alkaloid series; we are grateful to these colleagues for this information concerning their latest work. This approach and others are being actively pursued.

Experimental

General directions and the methods used for calculation of incorporations were published in Parts III[14b] and IV.[15c] The methods described there for proof of purity of the isolated alkaloids were also used in the present work. Early assays of radioactivity were carried out as reported in Part II,[26] but most followed the method described in Part VIII.[27]

Cultivation of Cephaelis ipecacuanha *plants and Administration of Labelled Precursors.*—The plants were grown in separate pots in a heated (>65°) and shaded glasshouse with steam supply and water spray, under conditions designed to simulate those of a South American rain forest. Radioactive precursors were introduced into the plants (1·5—3 yr. old) as aqueous solutions (generally 1—2 ml.) by means of an untreated cotton wick passed through the upper part of the stem. The plants were normally harvested 1—3 weeks (occasionally 5 weeks) after the start of the feeding experiment.

Extraction and Separation of the Alkaloids.—Five plants (1·5 yr. old; wet wt. 69 g.) were cut into small pieces (*ca.* 1 cm.) and then macerated with ethanol (200 ml.) in a Waring blender. The resultant suspension was poured into a glass column fitted with a filter pad, and the plant material was percolated first with ethanol (3 l.) and then with 1 : 1 aqeous ethanol (2·8 l.). The residue from evaporation of the latter extract was combined with the concentrated ethanolic extract (*ca.* 100 ml.) and water (200 ml.) was added before the ethanol was completely evaporated. Extraction of the aqueous suspension with ether (4 × 125 ml.) gave an organic solution which was shaken with aqueous 0·1M-potassium dihydrogen phosphate (3 × 100 ml.) and rejected. The phosphate solution and the original aqueous solution were combined, adjusted to pH 10 with solid potassium carbonate, and extracted with 1 : 4 (by vol.) chloroform–ether (5 × 250 ml.). The aqueous phase was adjusted to pH 5 and reserved for extraction of ipecoside.[28]

The combined organic solution was extracted with 0·4N-hydrochloric acid (5 × 200 ml.), each extract being back extracted with chloroform–ether. Basification of the combined acidic solution with potassium carbonate, extraction as before with chloroform–ether, and evaporation of the extracts to *ca.* 40 ml. afforded a solution of the total alkaloids. This was diluted with ether (250 ml.), and the solution was extracted with N-sodium hydroxide (4 × 150 ml.), each being rapidly back-extracted with ether–chloroform before being run into 3N-hydrochloric acid (220 ml.). Evaporation of the water-washed organic phase gave the non-phenolic alkaloids (124 mg.). Basification of the acidic solution with potassium carbonate and extraction with ether–chloroform afforded the phenolic alkaloids (514 mg.). The latter were chromatographed in 1 : 1 (by vol.) benzene–chloroform over alumina (neutral; activity III), to yield cephaeline (484 mg.), shown to be homogeneous by thin-layer chromatography on silica gel (solvent: 15% methanol in chloroform with 2 drops of 0·880 ammonium hydroxide for 50 ml. of solvent mixture). This material crystallised readily from moist ether but was used as such for conversion into emetine.

[25] A. R. Battersby and G. V. Parry, *Tetrahedron Letters*, 1964, 787.
[26] A. R. Battersby, R. Binks, and B. J. T. Harper, *J.*, 1962, 3534.
[27] A. R. Battersby, D. M. Foulkes, and (in part) R. Binks, *J.*, 1965, 3323.
[28] P. Bellet, *Ann. pharm. franç.*, 1954, **12**, 466.

Methylation of Cephaeline.—(*a*) A solution of cephaeline (606 mg.) in methanol (12 ml.) was treated with an excess of ethereal diazomethane [29] (from 6 g. of *N*-nitroso-*N*-methylurea). After the solution had been kept at room temperature for 20 hr., it was freed from diazomethane by a nitrogen stream and then worked for non-phenolic bases (546 mg.) as above. Thin-layer chromatography generally showed emetine to be the major alkaloid present but it was accompanied by another substance of as yet unknown nature which occasionally was the main product. This unknown material differed from *N*-methylemetine.[30]

(*b*) Trimethylanilinium chloride (125 mg.) in methanol (1 ml.) was treated with methanolic potassium hydroxide (1% by wt.; 5 ml.) and the precipitated salt was filtered off. The filtrate was mixed with a solution of cephaeline (247 mg.) in methanol (1·5 ml.) and then added during 15 min. to xylene (8 ml.) at 110—115° during which period much of the methanol distilled out. The temperature was then raised to 125—130° and held there for 30 min. after all the methanol had evaporated. More xylene (*ca.* 2 ml.) was added during this period to overcome gel formation. Phenolic (28 mg.) and non-phenolic (219 mg.) fractions were isolated as above; the latter consisted of pure emetine (thin-layer chromatography). This was converted into its dimethiodide [2] or into the *N*-phenylurea [3c] for counting. The urea was purified by chromatography in 3 : 7 (by vol.) chloroform–benzene on neutral alumina (Activity III) and crystallisation twice from aqueous methanol; in the example described, the product (193 mg.) had m. p. 216—218° (lit.,[3c] 220—221°).

N-*Methylemetine Perchlorate.*—Aqueous formaldehyde (40%; 3 ml.) was added to a solution of emetine (306 m.g) in formic acid (98%; 3 ml.), and the mixture was heated at 100° for 5 hr. Ether extraction of the basified solution afforded a resin which was dissolved in 0·05N-hydrochloric acid (30 ml.) and treated with an excess of sodium perchlorate. Recrystallisation of the percipitate from aqueous methanol afforded N-*methylemetine perchlorate* (344 mg.), m. p. 219—220° (Found: C, 49·8; H, 6·4. $C_{30}H_{44}Cl_2N_2O_{12},2H_2O$ requires C, 49·3; H, 6·6%). Singlet τ 7·5 (N–Me) in $CDCl_3$.

Kuhn–Roth Oxidation and Separation of Products.—(a) *Separation as acids.* The oxidation of emetine-*N*-phenylurea was carried out in the standard way; [31] the steam-distillate containing the volatile acids was titrated with 0·05N-sodium hydroxide and then adjusted with an excess to pH 11. The solution obtained by evaporation to near dryness was adjusted until just alkaline to phenolphthalein and evaporated to dryness.

Purified silica gel (100 g.) which had been dried at 120° for 48 hr. was thoroughly shaken with glycine buffer [32 g.; prepared by adjustment of a solution of glycine (1·88 g.) in water (50 ml.) to pH 2·0 with 2N-hyrochloric acid]. Part (40 g.) was packed as a slurry in chloroform into a glass column. The sodium salts, above, were dissolved in glycine buffer [0·5 ml.; prepared by adjustment of a solution of glycine (7·5 g.) in water (50 ml.) to pH 2·0], and the solution was adsorbed on to dry, unbuffered silica gel (2 g.) which was then packed on to the top of the main silica gel column. Elution with chloroform and titration of the fractions (5 ml.) with sodium hyroxide gave, after steam-distillation to remove indicator, sodium acetate and sodium propionate.

That the separation of acids was quantitative by this procedure was established in two experiments with synthetic mixtures of acetic and propionic acids to which were added, respectively, tracer quantities of sodium [1-^{14}C]acetate and sodium [1-^{14}C]propionate. Negligible activity appeared in the unlabelled acid in each case.

[*ethyl*-1-^{14}C]Stilbœstrol, of activity 4·6 × 10^6 dis. per 100 sec. per mmole (rel. activity 1·00), was subjected to this procedure to afford sodium acetate (2·28 × 10^6 dis. per 100 sec. per mmole; rel. activity 0·49; theory 0·50) and sodium propionate (2·17 × 10^6 dis. per 100 sec. per mmole; rel. activity 0·47; theory 0·50).

(b) *Separation as* p-*bromphenacyl esters* [with Dr. D. A. Yeowell]. The steam distillate from the oxidation of emetine-*N*-phenylurea (110—180 mg.) was titrated with 0·074N-lithium hydroxide (carbonate-free) to pH 8·5, and the solution was then evaporated to dryness under pure nitrogen. A solution of the residue in a few drops of water was treated with *p*-bromophenacyl bromide (1·05 equiv.) in ethanol (25 ml.), and the mixture was heated under reflux for 30

[29] P. Karrer, *Ber.*, 1916, **49**, 2057.
[30] F. H. Carr and F. L. Pyman, *J.*, 1914, **105**, 1591.
[31] *E.g.*, J. W. Cornforth, R. H. Cornforth, A. Pelter, M. G. Horning, and G. Popjak, *Tetrahedron*, 1959, **5**, 311.

min. in an atmosphere of pure nitrogen. The residue obtained by evaporation was chromatographed in benzene on silicic acid (4 g.; 100 mesh), the separation being controlled by thin-layer chromatography on silica gel with benzene as solvent. Combination of appropriate fractions and crystallisation of the products from light petroleum (b. p. 40—60°) afforded *p*-bromophenacyl acetate (16—25 mg.), m. p. 84—85°, and *p*-bromophenacyl propionate (16—25 mg.), m. p. 61—62°.

Schmidt Degradation of Acetic Acid [with Dr. J. B. TAYLOR].—This reaction was carried out by the procedure of Arigoni,[32] to afford methylamine which, after steam-distillation, was converted into *N*-methylbenzamide by benzoyl chloride in a micro-adaptation of the Schotten–Baumann method. The derivative crystallised from benzene–light petroleum (b. p. 60—80°), m. p. 79—81°.

Degradation of Cephaeline from Plants fed with [2-^{14}C]*Tyrosine*.—The reaction sequence is a known one.[2] The diluted cephaeline (872 mg.) was methylated (diazomethane) to give emetine (585 mg.) which was further diluted with radio-inactive emetine and converted into the hydrobromide [30] (5·12 g.). The following products and yields were then obtained without further dilution. Emetine dimethiodide (XI), 6·11 g.; emetinemethine, 3·6 g.; tetrahydroemetinemethine perchlorate, 4·33 g.; tetrahydroemetinemethine dimethiodide, 1·53 g.; de-*N*(a)-tetrahydroemetinemethine perchlorate (XII), 0·64 g.; de-*N*(a)-tetrahydroemetinemethine methiodide, 2·69 g.; de-*N*(a)-hexahydroemetinebismethine picrate (XIV), 1·64 g.; 6-ethylveratric acid (ring A), 75 mg.; 6-ethylveratric acid (ring F), 55 mg.

Purification of the crude 6-ethylveratric acid was achieved by chromatography in 1 : 4 (by vol.) chloroform–benzene over silica gel, recrystallisation of the crystalline fractions from di-isopropyl ether, and sublimation of the product at 160°/10 mm., to give the above quantities, m. p. 146—147°.

The Schmidt reaction on 6-ethylveratric acid was carried out as previously [10] save that chloroform was substituted for benzene. Assay of the barium carbonate was carried out as in Part II.[26]

*Attempted Oxidation of De-*N(a)*-Hexahydroemetinemethine* (as XII, double bond reduced).—The methine (807 mg.) was oxidised in " stabilised " acetone (30 ml.) with aqueous barium permanganate under the conditions used above to afford 6-ethylveratric acid from the unsaturated methines. The same work-up procedure was used to afford an amorphous acidic fraction (7 mg.) from which no 6-ethylveratric acid could be isolated.

Characterisation and Cleavage of N*-Acetylemetinemethine* (XV).—A further part of the active emetine (360 mg.) derived from the experiment with [2-^{14}C]tyrosine was converted by the improved method [33] (cf. ref. 34) into the amorphous *N*-acetylemetinemethine. This was chromatographed on neutral alumina (activity III) in 1 : 9 (by vol.) chloroform–benzene, and the appropriate fractions afforded almost pure methine (266 mg.) which was treated in ethanol with picric acid (120 mg.). Recrystallisation of the precipitate from ethanol afforded N-*acetylemetinemethine picrate* (330 mg.), m. p. 138—139° (decomp.) (Found: C, 59·5; H, 6·2; N, 9·3. $C_{38}H_{47}N_5O_{12}$ requires, C, 59·6; H, 6·1; N, 9·2%).

A solution of the picrate (150 mg.) in chloroform was passed down a short column of alumina. Evaporation of the percolate afforded the pure methine which was cleaved by treatment at room temperature with osmium tetroxide (6 mg.) and sodium metaperiodate (130 mg.) in purified t-butyl alcohol (6·5 ml.) and water (5 ml.) for 2·5 hr. More metaperiodate (20 mg.) was then added, and the stirred reaction mixture kept for a further 2·5 hr. before being worked for formaldehyde by the method developed below for *N*-acetyltetrahydroemetinebismethine. There was obtained formaldehyde dimethone (36 mg.), m. p. and mixed m. p. 188°. A blank run yielded no formaldehyde dimethone.

The same cleavage conditions were applied to *N*-acetyldihydroemetinemethine recovered from the corresponding picrate; [33] no formaldehyde was produced.

N-*Acetyltetrahydroemetinebismethine Methopicrate* (as XXIV).—A solution of *N*-acetyldihydroemetinebismethine [33] (439 mg.) in ethanol was shaken with platinum oxide (97 mg.) and hydrogen at room temperature and pressure; uptake ceased after 1 mol. of hydrogen had been absorbed. The recovered base was chromatographed over alumina and eluted with 3 : 1 (by vol.) benzene–chloroform, to afford a gum. This was heated under reflux in methanol (10 ml.) with methyl iodide (5 ml.) for 7 hr., and the solution was then evaporated to a gum which

[32] D. Arigoni and co-workers, unpublished work.
[33] A. R. Battersby, R. Binks, and T. P. Edwards, *J.*, 1960, 3474.
[34] A. Ahl and T. Reichstein, *Helv. Chim. Acta*, 1944, **27**, 366; M. Pailer, *Monatsh.*, 1948, **79**, 127.

could not be crystallised. Part of this methiodide (30 mg.) was treated in aqueous ethanol with a solution of sodium picrate (8·8 mg.), and the precipitate was recrystallised twice from aqueous acetone to give N-*acetyltetrahydroemetinebismethine methopicrate*, m. p. 92—94° (Found, on material dried at 54°: C, 60·0; H, 7·1. $C_{40}H_{55}N_5O_{12}$ requires C, 60·2; H, 6·95%).

Preparation and Cleavage of N-*Acetyltetrahydroemetinetrismethine* (XXV).—The foregoing methiodide (510 mg.) was converted into the methohydroxide with moist silver oxide as usual [2,33] and the solution was evaporated to low volume. Potassium hydroxide (4 g.) was then added, the solution was evaporated to dryness, and the residue heated at 112° for 4 hr. at 12 mm. Ether and water were then added and the ether-soluble portion (357 mg.) was heated with acetic anhydride (5 ml.) at 60° for 1 hr. After evaporation of the acetic anhydride, the residue was partitioned between ether (200 ml.) and 2N-hydrochloric acid (30 ml.). Evaporation of the ethereal solution gave the neutral fraction (158 mg.). Basification of the acidic solution and extraction thrice with ether afforded the basic fraction (185 mg.) which was converted into its methiodide and treated again as above to yield more neutral material (70 mg.). The combined neutral fractions were purified by chromatography on alumina in 9 : 1 (by vol.) benzene–chloroform, to give *N*-acetyltetrahyroemetinetrismethine (XXV) as a gum (165 mg.); this was homogeneous by thin-layer chromatography ($\nu_{max.}$ in CCl_4, 893 cm.$^{-1}$; 2-proton broad singlet at τ 5·08, $>C{=}CH_2$).

Sodium (5 g.) was added to t-butyl alcohol (300 ml.), and the mixture heated under reflux for 1 hr. The alcohol was then distilled and used immediately to prepare a solution (25 ml.) of osmium tetroxide (0·1 g.). The foregoing trismethine (135 mg.) in purified t-butyl alcohol (5 ml.) and water (5 ml.) was stirred with osmium tetroxide (1·5 ml. of above solution) and sodium metaperiodate (130 mg.), the latter being added in portions during 50 min. A further addition of metaperiodate (20 mg.) was made after 19 hr. After a total period of 24 hr.. a saturated aqueous solution of arsenious oxide (50 ml.) was added and the mixture was extracted thrice with ether to afford a gum (120 mg.) and an aqueous solution (*A*). Chromatography of a solution of the gum in 9 : 1 benzene–chloroform on alumina and crystallisation of the pure fractions from ether yielded the *ketone* (XXVI) (48 mg.), m. p. 113—114° (Found: C, 70·7; H, 8·1. $C_{30}H_{41}NO_6$ requires C, 70·4; H, 8·1%), $\nu_{max.}$ 1720 cm.$^{-1}$; no signal at τ 5·08.

The aqueous solution *A* was adjusted to pH 9—10 with potassium carbonate, dimedone (0·3 g.) was added, and after 10 min. the pH was adjusted to 6. After the mixture had been kept overnight at room temperature, the solid (44 mg.) was collected and purified by filtration in chloroform through a short column of alumina. Recrystallisation of the product from ethanol gave formaldehyde dimethone (31 mg.), m. p. 191·5—192°.

Hydrogenation of the tetrahyrotrismethine (XXV) as above (0·96 mol. uptake) and chromatography of the product afforded *N*-acetylhexahydroemetinetrismethine; there was no peak at 893 cm.$^{-1}$ and no signal at τ 5·08. This product was treated with osmium tetroxide and sodium metaperiodate as above; no formaldehyde dimethone was produced (recovery of starting material, 89%).

Preparation and Cleavage of the Diol (XVII).—A solution of *N*-acetyldihydroemetinebismethine (XVI) (349 mg.) in dry ether (3 ml.) was treated with a solution of osmium tetroxide (0·2 g.) in ether (4 ml.) containing pyridine (0·15 ml.) and the mixture was kept at room temperature for 24 hr. The ether layer was then removed from the precipitate, the latter was washed with ether, and the combined ethereal solutions were reserved. The osmate ester was heated under reflux for 5 hr. with ethanol (15 ml.) and a solution of sodium sulphite (1·5 g.) in water (7·5 ml.). After the cooled suspension had been mixed with ethanol (20 ml.), the solid was filtered off and the filtrate evaporated to dryness. That part (336 mg.) of the residue which was soluble in ethanol gave one slow-running spot on thin-layer chromatograms; it was free from starting material.

The ethereal solution above was treated as before with osmium tetroxide (0·1 g.) and by the same procedure yielded more diol (39 mg.).

Lead tetra-acetate (340 mg.) was added to a solution of the combined diol fractions above (375 mg.) in dry benzene (40 ml.) and, after the mixture had been kept at room temperature for 15 hr., the solution was decanted from the precipitate. The latter was washed with benzene (3 × 10 ml.) and the combined solutions in benzene were evaporated to dryness. The residue was worked for neutral (plus acidic) material through N-hydrochloric acid and ether as usual, and the ethereal extracts afforded 6-ethylveratraldehyde (126 mg.), shown by thin-layer chromatrography to contain a little 6-ethylveratric acid.

The aqueous layer and washings were concentrated to *ca.* 15 ml., basified with potassium carbonate, and extracted with chloroform (4 × 5 ml.), to yield the amino-aldehyde fraction (XIX) as a gum.

A solution of all the crude 6-ethylveratraldehyde above in 1 : 1 pyridine–water (3 ml.) was stirred vigorously at 60° while aqueous sodium hydroxide (32% w/v; 0·5 ml.) was added. The addition to this emulsion of 30% hydrogen peroxide was started immediately, and the temperature was raised to 80° during 30 min.; the hydrogen peroxide (total 25 ml.) was added during 3 hr. Ether extraction of the cooled solution gave unchanged aldehyde (59 mg.) which was re-treated as before. The alkaline solutions from both experiments were acidified and extracted with ether, to afford 6-ethylveratric acid (109 mg.), which was purified by sublimation and recrystallisation from di-isopropyl ether as earlier, m. p. 146—147°.

5,6-*Dimethoxy*-3-*methylphthalide* (XX).—When crude 6-ethylveratraldehyde (47 mg.), as in foregoing experiment, was oxidised in pyridine (3 ml.) with aqueous potassium permanganate (38·9 mg. in 12 ml.) at 20° for 2 hr., a mixture was formed. Fractionation afforded crystals (*ca.* 15 mg.) which were identified by comparison with material prepared as follows.

A solution of 6-vinylveratric acid [18] (254 mg.) in a mixture of concentrated suphuric acid and formic acid (1 : 9 by vol.; 15 ml.) was kept at 20° for 60 hr. and then poured into water. This solution was extracted with 4 : 1 ether–chloroform, and the combined extracts were washed with alkali before being evaporated to a gum (*B*) (81 mg.). Acidification of the alkaline solution and extraction with ether–chloroform afforded a mixture of the desired phthalide and other products. This mixture was sublimed at 120°(bath)/11 mm.; the early fractions crystallised readily and were identical with gum *B*, with which they were combined. Crystallisation from di-isopropyl ether gave 5,6-dimethoxy-3-methylphthalide [16] (81 mg.), m. p. 120—121° (Found: C, 63·7; H, 5·7. Calc. for $C_{11}H_{12}O_4$, C, 63·5; H, 5·8%). This was identical with the foregoing product.

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