Deacetylisoipecoside: the Key Intermediate in the Biosynthesis of the Alkaloids Cephaeline and Emetine

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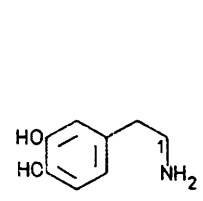
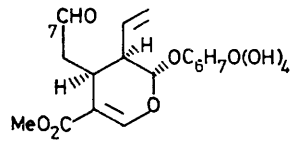
Summary In contrast to previous assumptions, the precursor of the monoterpenoid ipecac alkaloids cephaeline (**6**) and emetine (**5**), which arise from the condensation of dopamine (**1**) and secologanin (**2**), is α-deacetylisoipecoside (**3a**) and not its β-epimer deacetylipecoside (**4a**); the latter is the precursor of ipecoside (**4b**) and alangiside (**7a**) both of which possess the β-configuration.

THE nitrogenous glucoside ipecoside[1] has been found to occur in *Cephaelis ipecacuanha* and its structural elucidation[2] gave a clue as to the biosynthesis of the monoterpenoid ipecac and indole alkaloids. On the basis of chemical and spectroscopic evidence, the stereochemistry at C-1 was reported to have the α-configuration. *X*-Ray analysis[3] of the natural product, as its crystalline dimethyl ether (**4c**) revealed, however, that the glucoside has its C-1 hydrogen atom in the β- rather than the α-configuration. With this point established for (**4b**), it followed, from the previous correlations in the indole alkaloid series,[4] that the C-3 configuration for the (at that time assumed) biosynthetic precursor vincoside (**11**) is β and for strictosidine (isovincoside) (**10**) is α. Furthermore, biosynthetic experiments[5] using labelled (**3a**) with the 1-α- and (**4a**) with the 1-β-configuration demonstrated the exclusive incorporation of deacetylipecoside (**4a**) into ipecoside (**4b**) as well as the alkaloids (**5**) and (**6**), the latter having the α-configuration. These findings implied for both families of alkaloids, the isoquinoline type found in *Cephaelis* and *Alangium*[6] as well



(1)



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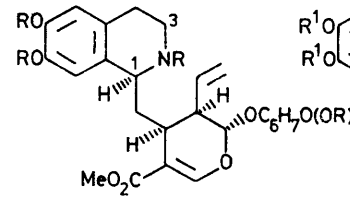
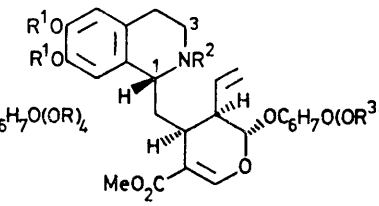


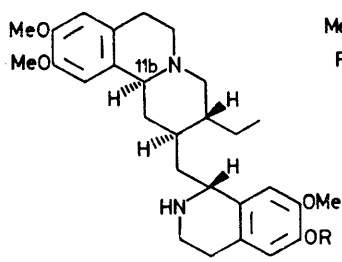
(3)

a; R = H
b; R = Ac

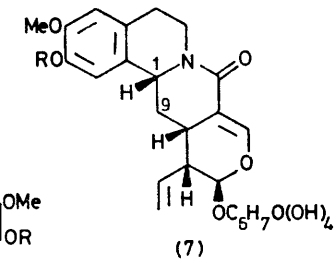


(4)

a; $R^1 = R^2 = R^3 = H$
b; $R^1 = R^3 = H, R^2 = Ac$
c; $R^1 = Me, R^2 = Ac, R^3 = H$
d; $R^1 = R^2 = R^3 = Ac$

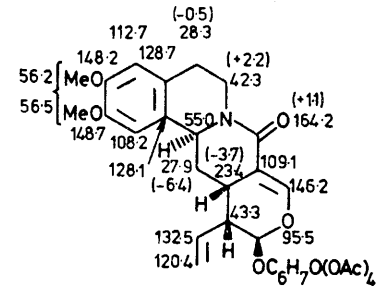


(5) R = Me
(6) R = H

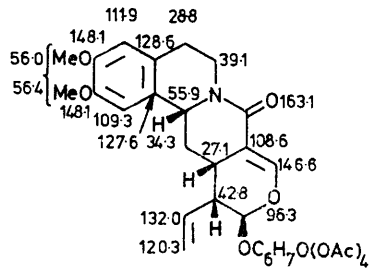


(7)

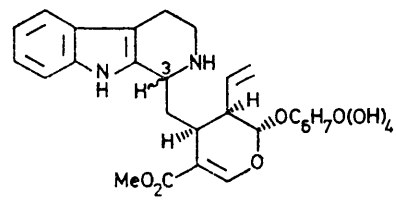
a; R = H
b; R = Me



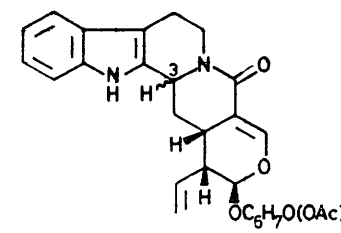
(8)



(9)



3-H
(10) α
(11) β



3-H
(12) α
(13) β

as the monoterpenoid indole alkaloids, that the immediate biosynthetic precursors had the β- rather than the α-configuration. This was concluded despite the fact that the α-configuration would have represented the correct stereochemical relationship of the precursors to the products and suggested the occurrence of an inversion from β to α during the biosynthetic reaction sequences, a reaction of much controversy and confusion.[7] Recently we were able to prove, by both tracer feeding and enzymatic studies, that (**10**), with the 3α(*S*)-configuration, rather than (**11**) is the common precursor for the biosynthesis of the 3α- and the 3β-monoterpenoid indole alkaloids.[8,9] This finding made it necessary to reinvestigate the biosynthesis of the ipecac alkaloids and of ipecoside (**4b**). The configuration of ipecoside (**4b**) was first reconfirmed. It was isolated[10] from dried plant material and c.d. measurements on the hexa-acetate (**4d**) showed λ_{max} 274·2 nm ($\Delta\epsilon - 0{\cdot}638$) and λ_{max} 249·8 mn ($\Delta\epsilon + 1{\cdot}063$). Comparison with model compounds of known absolute configuration showed (**4b**) to have a β-configuration.[11]

lamarckii; the latter contains alangiside[16] (**7a**) and cephaeline (**6**). The compounds under investigation (**4b**), (**5**), (**6**), and (**7a**) were identified and analysed by t.l.c. co-chromatography, radioscanning, dilution with unlabelled material, crystallization to constant specific activity, and in some cases formation of derivatives. The essential experimental data and results from the feeding experiments are shown in the Table.

Deacetylipecoside (**4a**) is exclusively and specifically incorporated into the nitrogenous glucosides (**4b**) in *Cephaelis* and (**7a**) in *Alangium*, both having the β-configuration. No incorporation into the alkaloids (**5**) and (**6**) has been observed. In contrast, the epimer with the α-configuration, deacetylisoipecoside (**3a**) is the true precursor of the ipecac alkaloids (**5**) and (**6**) with the α-configuration in both plants, and no radioactive label was found in the glucoside fraction with the β-configuration. It is important to note that in the α and β series of the phenolic products, the original tritium atom at C-1 of the precursors (**3a**) and (**4a**) is retained, whereas in the indole series the

TABLE

Tracer experiments on *Cephaelis ipecacuanha* and *Alangium lamarckii*

Precursor (Stereochemistry at C-1)	Plant	Incorporation/% (**4b**) (1-β)	(**7a**)[a] (1-β)	(**6**) (11b-α)	(**5**) (11b-α)
[1-^{3}H, 3-^{14}C]-(**4a**, β) (^{3}H:^{14}C ratio 5·94)	*Cephaelis*	3·23 (6·00)	—	<0·005	<0·005
[1-^{3}H, 3-^{14}C]-(**3a**, α) (^{3}H:^{14}C ratio 5·60)	*Cephaelis*	<0·005	—	6·66 (5·66)	0·33 (5·49)
[1-^{3}H, 3-^{14}C]-(**4a**, β) (^{3}H:^{14}C ratio 6·02)	*Alangium*	—	38·83 (6·06)	<0·005	—
[1-^{3}H, 3-^{14}C]-(**3a**, α) (^{3}H:^{14}C ratio 5·01)	*Alangium*	—	<0·005	0·16 (5·65)	—

[a] Isolated as O-methylalangiside (**7b**).

The role of the suspected precursors was studied by feeding either [1-^{3}H, 3-^{14}C]-(**3a**) or (**4a**) to *C. ipecacuanha* and *Alangium lamarckii*. The precursors were obtained by condensing [1-^{14}C]dopamine (**1**) with [7-^{3}H]secologanin[9] (**2**) and separating the epimers by chromatography.[10] Unequivocal assignments of the epimers to the α- or β-configuration was achieved by converting (**3a**) and (**4a**), as their *OO*-dimethyl derivatives, into their respective lactams.[12,13] Acetylation and n.m.r. spectroscopic examination ($CDCl_3$, Me_4Si, 270 MHz) proved (**9**) [H-1-β, δ 4·72 (dd, *J* 12 and 3 Hz), H-9-α, δ 1·45 (ddd, *J* 13, 13, and 12 Hz)] to be identical to *O*-methylalangiside tetra-acetate.[13] Compound (**8**) [H-1-α, δ 4·64 (dd, *J* 5 Hz), H-9-β, δ 2·23 (ddd, *J* 14, 5, and 5 Hz)] showed in addition a characteristic anomalous acetate signal at δ 1·57. The latter signal has also been reported for the 3α-indole homologue strictosamide tetra-acetate (**12**)[8b,14] but is not observed with the corresponding β-derivative (**13**). C.d. measurements of the hexa-acetates (**3b**) and (**4d**) were in agreement with the n.m.r. results and (**4d**) was also in all other respects identical to the hexa-acetate of natural ipecoside. An exhaustive ^{13}C n.m.r. analysis ($CDCl_3$, Me_4Si, 20 MHz) of (**8**) and (**9**) again confirmed their stereochemistry by comparison with the ^{13}C n.m.r. spectra of the respective lactams (**12**) and (**13**) of the indole series.[15]

The incorporation studies were conducted using either leaves or seedlings of *C. ipecacuanha* or apical cuttings of *A.*

formation of the 3β-alkaloids from the 3α-precursor (**10**) proceeds with loss of the corresponding hydrogen atom.[9]

These results prove, that contrary to previous assumptions, no epimerization of the precursors in the biosynthesis of ipecac alkaloids is involved. The α-epimer deacetylisoipecoside (**3a**) is the key intermediate in the formation of the multitude of ipecac alkaloids represented here by cephaeline (**6**) and emetine (**5**). The C-1 β-epimer, deacetylipecoside (**4a**), is either acetylated in the plant, to give ipecoside (**4b**), or its methyl ester group is hydrolysed and subsequently transformed to alangiside (**7a**); however, the β-configuration is retained. Both metabolites with the β-configuration seem to be, in these plants, at a biosynthetic 'dead end' and metabolically inert. Thus, the *N*-acetylation reaction may be regarded as a metabolic inactivation of an intermediate with 'wrong' stereochemistry as has been observed, for instance, in the case of D-tryptophan which is rendered inactive either by acetylation or by malonylation.[17] The natural occurrence of both epimers (**3a**) and (**4a**) in the isoquinoline series is quite different from the situation in indole alkaloid biosynthesis,[8b,9] where only the α-epimer is formed, but can serve also as precursor of alkaloids with the β-configuration.[9]

The pathway suggested here involves the condensation of dopamine (**1**) and secologanin (**2**) with the formation of both deacetylipecoside (**4a**) and deacetylisoipecoside (**3a**). While the former is metabolically inactivated by acetylation to

give ipecoside (**4b**) or hydrolysed to yield alangiside (**7a**), the α-epimer (**3a**) is further transformed to several monoterpenoid isoquinoline alkaloids including cephaeline (**6**) and emetine (**5**).

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