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The biosynthesis of β-carboline and quinolizidine alkaloids of *Alangium lamarckii*

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Abstract

The incorporation of tryptamine, dopamine, *N*-deacetylisoipecoside, *N*-deacetylipecoside into alangimarckine, deoxytubulosine and ankorine and of strictosidine and vincoside into alangimarckine and deoxytubulosine in young *Alangium lamarckii* Thw. (Alangiaceae) has been studied and specific utilisation of *N*-deacetylisoipecoside demonstrated. Parallel experiments with nordeoxytubulosine and deoxytubulosine suggested that *O*-methylation precedes condensation of protoemetine with tryptamine and further the reduction of ethylene side chain takes place before condensation. Hydroxylation at C-8 in the *trans*-quinolizidine moiety is the terminal step in the biosynthesis of alangimarckine.

Keywords: *Alangium lamarckii* Thw.; Alangiaceae; Antimicrobial; Biosynthesis; Alkaloids; Deoxytubulosine; Alangimarckine; Ankorine

1. Introduction

The β-carboline alkaloids, deoxytubulosine (**17**) (Battersby et al., 1965), tubulosine (**19**) (Pakrashi, 1964) and alangimarckine (**20**) (Battersby et al., 1966), the quinolizidine alkaloid ankorine (**6**) (Battersby et al., 1966; DasGupta, 1965) and various nitrogenous glucosides (Itoh et al., 1998) are produced by *Alangium lamarckii* Thw. a plant that has potent antimicrobial activity (Openshaw and Whittaker, 1969). Recently two new alkaloids namely 1′,2′-dehydrotubulosine and alangine have been isolated from *A. lamarckii* plant (Itoh et al., 1999, 2000). The alkaloidal extract of leaves has shown mild adrenolytic, antispasmodic, hypotensive and anticholinesterase activity (Sanyal et al., 1965, 1966). An amorphous yellow alkaloid (AL 60) has shown biphasic action on blood pressure of intact cats (Datta and Pakrashi, 1962a). The base also exerted spasmodic and spasmolytic effects in smooth muscles (Datta and Pakrashi, 1962b). A strong binding of deoxytubulosine with DNA has been shown by its absorption and emission spectra (Venkatachalam et al., 1994). The dihydrofolate reductase and cell growth activity inhibition was also reported by the base (Rao and Venkatachalam, 1999).

We report for the first time, the results of incorporation of labelled tryptamine, dopamine, *N*-deacetylisoipecoside and *N*-deacetylipecoside into alangimarckine, deoxytubulosine and ankorine and of strictosidine (**11**) and vincoside (**12**) into alangimarckine and deoxytubulosine in young *A. lamarckii* Thw. (Alangiaceae).

2. Results and discussions

The biosynthesis of deoxytubulosine (**17**) and alangimarckine (**20**) is expected to be from deacetylisoipecoside (**1**). Enzymic hydrolysis of **1** and opening of ring C could furnish the dialdehyde (**3**) in which at least the stereochemistry at C-1 and C-4′ is retained. Condensation of the appropriate aldehyde function with the secondary amino function, followed by reduction of the corresponding iminium intermediate, could give rise to the protoemetine skeleton (**4**). Compound **4** would then react with tryptamine (**15**) (derived from tryptophan) to yield the key intermediate *O*-nordeoxytubulosine (**16**). Hydroxylation of **16** at C-8 could give a compound of the type **18** which by selective *O*-methylation of the *ortho*-phenolic hydroxyl group at C-9 and C-10, could

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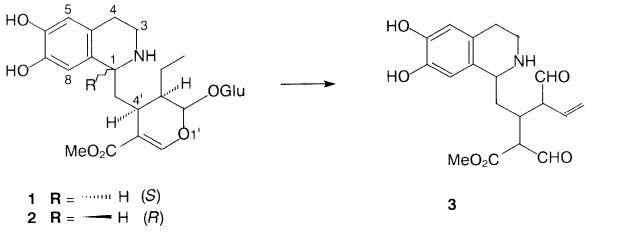
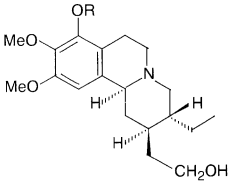
finally give alangimarckine (**20**). However, *O*-methylation of phenolic hydroxyl groups in **4** could give rise to protoemetine nucleus of the type **5** which undergoes condensation with **15** to give **17**. Hydroxylation at C-8 in **17** could finally give **20**. Alternately, *O*-methylation of the *ortho*-phenolic hydroxyl groups at C-9 and C-10 in the key intermediate *O*-nordeoxytubulosine (**16**) could give deoxytubulosine (**17**) which on selective hydroxylation at C-8 could finally yield alangimarckine (**20**). On the other hand **17** and **20** could also be formed from strictosidine (**11**), the universal precursor for terpenoid indole alkaloids (Battersby, 1967; Battersby et al., 1968a,b, 1969a; Mattes et al., 1975). Hydrolysis of **11** followed by opening of the pyran ring and decarboxylation could afford the dialdehyde (**13**). Condensation of compound **13** with dopamine (**14**) would generate the *trans*-quinolizidine moiety (**16**) which could give rise to **17** and **20** as described above. Reduction of ethylene side chain, however, can take place at any stage of the biosynthesis of deoxytubulosine.

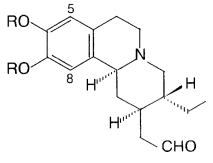


1 R = ····· H (*S*)
2 R = ——— H (*R*)

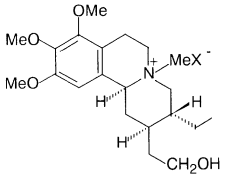
3



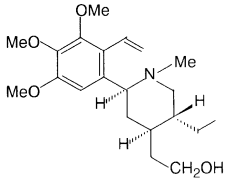
6 R = H
7 R = Me



4 R = H
5 R = Me

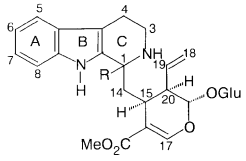


8 X = I
9 X = OH

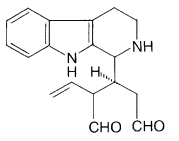


10

The quinolizidine alkaloid, ankorine (**6**) could be formed from the key intermediate **4** by hydroxylation at C-5 followed by selective *O*-methylation of the phenolic hydroxyl groups at C-6 and C-7 positions and reduction of the aldehyde group into the corresponding secondary alcoholic function. Alternately, *O*-methylation of phenolic hydroxyl groups in **4** could follow hydroxylation of the *O*-methyl derivative (**5**).



11 R = H (*S*)
12 R = H (*R*)



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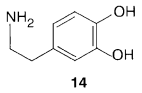

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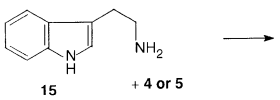
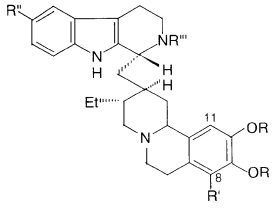
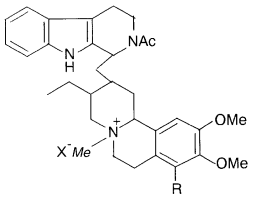


15 + **4 or 5**





16 R = R' = R" = R"' = H
17 R = Me; R' = R" = R"' = H
18 R = R"= R"' = H; R' = OH
19 R = Me; R' = R'" = H; R" = OH
20 R = Me; R' = OH; R" = R'" = H
21 R = Me; R' = R" = H; R'" = Ac
22 R = Me; R' = OAc; R" = H; R'" = Ac

23 R = H ; X = I
24 R = H ; X = OH
25 R = OMe ; X = I
26 R = OMe ; X = OH

Initial feeding of (L)-[U^{14}-C]tyrosine (Table 1, experiment 1) to young *A. lamarckii* Thw. plants demonstrated that deoxytubulosine (**17**), alangimarckine (**20**) and ankorine (**6**) are being biosynthesized by the young plants at the time of feeding. Feeding of tryptamine (**15**) (experiment 2) and dopamine (**14**) (experiment 3) revealed that both the amines were being used by the young plants to form deoxytubulosine (**17**) and alangimarckine (**20**) whereas only **14** was used by the plants to form ankorine (**6**).

Feeding of a mixture of [3-^{14}C]strictosidine (**11**) and [3-^{14}C]vincoside (**12**) (experiment 4) demonstrated that neither **11** nor **12** was used by the young plants to form deoxytubulosine (**17**) and alangimarckine (**20**).

The epimeric monoterpenoid isoquinoline glucosides, *N*-deacetylisoipecoside (**1**) and *N*-deacetylipecoside (**2**) have α- and β-configuration at C-1 respectively. Deoxytubulosine (**17**), alangimarckine (**20**) and ankorine (**6**) have α-configuration at C-11b corresponding to position C-1 of the epimeric monoterpenoid isoquinoline glucoside. *N*-Deacetylisoipecoside (**1**) having α-configuration at C-1 could be converted into **6**, **17** and **20** without any change in configuration at the corresponding positions. On the other hand if young *A. lamarckii* Thw. chose *N*-deacetylipecoside (**2**) having β-configuration at C-1 to form **6**, **17** and **20**, an inversion of configuration at C-1 must occur during the biotransformation.

Parallel feeding of [3-^{14}C]*N*-deacetylisoipecoside (**1**) (experiment 5) and [3-^{14}C]*N*-deacetylipecoside (**2**) (experiment 6) demonstrated that the stereo-specificity is maintained in the biosynthesis of **6**, **17** and **20**. The

Table 1
Tracer experiments on *Alangium lamarckii* Thw.

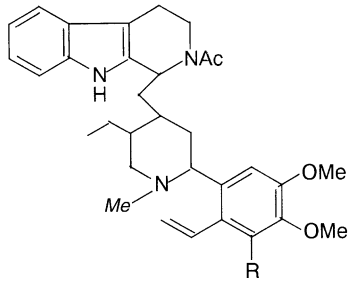
Expt. Precursor fed	Incorporation (%) into		
	Deoxytubulosine (**17**)	Alangimarckine (**20**)	Ankorine (**6**)
1 (L)-[U-^{14}C]Tyrosine	0.20	0.0142	0.012
2. [1-^{14}C]Tryptamine (**15**)	0.16	0.175	–[a]
3. [1-^{14}C]Dopamine (**14**)	0.424	0.175	0.12
4 [3-^{14}C]Strictosidine (**11**) and [3-^{14}C]vincoside (**12**)	0.002	0.0028	–[a]
5. [3-^{14}C]*N*-Deacetylisoipecoside (**1**)	3.218	11.0	0.213
6. [3-^{14}C]*N*-Deacetylipecoside (**2**)	0.18	0.88	0.02
7. [Aryl-^{3}H]Nordeoxytubulosine (**16**)	0.003	0.063	–[a]
8. [Aryl-^{3}H]Deoxytubulosine (**17**)	–[a]	1 .23	–[a]

[a] Incorporation not detected.

former was incorporated with higher efficiency than the latter. The incorporation of *N*-deacetylipecoside (**2**) into **6**, **17** and **20** was probably due to impurity of *N*-deacetylisoipecoside (**1**).

Biosynthetic ankorine (**6**) derived from [3-^{14}C]*N*-deacetylisoipecoside (**1**) (experiment 5) was treated with an excess of ethereal CH_2N_2 to afford *O*-methylankorine (**7**) with the same molar radioactivity as the parent base. Treatment of **7** with MeI gave *O*-methylankorine methiodide (**8**) which was passed through a column of freshly generated amberlite resin (IR-410; OH^- form) to give the corresponding methohydroxide (**9**). Hofmann degradation of **9** afforded the *O*-methylankorinemethine (**10**). Ozonolysis of **10** gave radioactive formaldehyde (dimedone derivative, 90% of original activity).

Biosynthetic deoxytubulosine (**17**) derived from [3-^{14}C] *N*-deacetylisoipecoside (**1**) (experiment 5) was treated with Ac_2O/pyridine to give *N*-acetyldeoxytubulosine (**21**) with essentially the same molar radioactivity as the parent base. Treatment of the radioactive **21** with MeI yielded *N*-acetyldeoxytubulosine methiodide (**23**) which was converted into its methohydroxide (**24**) by IR-410 anion exchange resin. Hofmann degradation of compound **24** yielded the methine (**27**) with essentially no loss of radioactivity. Ozonolysis of **27** gave radioactive formaldehyde (dimedone derivative, 87% of the original activity).



27 **R** = H
28 **R** = OMe

Biosynthetic alangimarckine (**20**) derived from [3-^{14}C]*N*-deacetylisoipecoside (**1**) (experiment 5) was treated with Ac_2O/pyridine to give *N,O*-diacetylalangimarckine (**22**) with essentially the same molar radioactivity as the parent base. Treatment of **22** with MeI in the presence of MeONa yielded *N*-acetyl-*O*-methylalangimarckine methiodide (**25**) which was converted into its methohydroxide (**26**) by IR-410 anion exchange resin. Hofmann degradation of **26** gave the methine (**28**) with essentially no loss of radioactivity. Ozonolysis of **28** gave radioactive formaldehyde (dimedone derivative, 82% of the original activity).

The intermediacy of deacetylisoipecoside in the biosynthesis of these bases is further supported (i) by the isolation of three tetrahydroisoquinoline monoterpene glucosides from *A. lamarckii* plant with the same absolute configuration as of deacetylisoipecoside (Itoh et al., 1994, 1995) and (ii) by the detection of enzyme activity responsible for the formation of deacetylipecoside and deacetylisoipecoside by the condensation of dopamine with secologanin in the cell free extract of *A. lamarckii* plant (De-Eknamkul et al., 1997).

O-Methylation of nordeoxytubulosine (**16**) could give deoxytubulosine (**17**). Alangimarckine (**20**) could be formed from **17** by nuclear hydroxylation at C-8. Alternately, hydroxylation could occur at nordeoxytubulosine (**16**) stage first, followed by selective *O*-methylation of the *ortho*-phenolic hydroxyl groups at C-9 and C-10 to yield **20**. Parallel feeding experiments with [aryl-^{3}H]-nordeoxytubulosine (**16**) (experiment 7) and [aryl-^{3}H]-deoxytubulosine (**17**) (experiment 8) revealed that **17** was incorporated about 20 times more efficiently than **16** into **20**, and **16** was poorly metabolized by the plants to form **17**, demonstrating thus, that, *O*-methylation of protoemetine nucleus (**4**) precedes its condensation with tryptamine (**15**) in the biosynthesis of alangimarckine (**20**) and further the reduction of ethylene side chain occurs before condensation. Hydroxylation at C-8 in transquinolizidine moiety is the terminal step in the biosynthesis of alangimarckine (**20**).

3. Conclusion

The foregoing experiments support the following sequence for the biosynthesis of ipecac-β-carboline alkaloids deoxytubulosine (**17**), alangimarckine (**20**) and the quinolizidine alkaloid ankorine (**6**) in young *A. lamarckii* Thw. plants.

Tyrosine→dopamine (**14**) + secologanin→*N*-deacetylisoipecoside (**1**)→deoxytubulosine (**17**)→alangimarckine (**20**). Tyrosine→dopamine (**14**)→*N*-deacetylisoipecoside (**1**)→ankorine (**6**).

Further, poor incorporation of nordeoxytubulosine as compared to deoxytubulosine supported that the condensation of tryptamine takes place with methylated protoemetine.

4. Experimental

Melting points were taken in sulfuric acid bath and are uncorrected. The ^{1}H NMR spectra were recorded on a Perkin Elmer P-30 (90 MHz) spectrometer. Mass spectra were recorded on a Jeol JMS D 300 spectrometer at an ionization energy of 70 eV. Infrared spectra were recorded on a Perkin Elmer Lambda spectro-photometer.

For general directions (counting method and labelling of precursors etc.) see earlier papers in the series (Bhakuni et al., 1977, 1979).

4.1. Synthesis and labelling of precursors

[1-^{14}C]3,4-Dihydroxyphenethylamine (dopamine) (**14**) was prepared by a known procedure (Bhakuni and Jain, 1981).

4.2. [1-^{14}C]Tryptamine (**15**)

Gramine was heated with MeI to give 3-dimethylaminomethylindole methiodide (Schramm, 1951) which on treatment with [^{14}C]KCN furnished 3-[1-^{14}C]acetonitrile indole. [1-^{14}C]Tryptamine (**15**) was obtained by catalytic reduction of 3-[1-^{14}C]acetonitrile indole (Terntiv et al., 1959).

4.3. [3-^{14}C]Strictosidine (**11**) and (3-^{14}C]vincoside (**12**)

Secologanin was condensed with [1-^{14}C]tryptamine (**15**) according to the standard procedure (Battersby et al., 1969b; Nagakura et al., 1978) to yield a mixture of [3-^{14}C]strictosidine (**11**) and [3-^{14}C]vincoside (**12**) which was used as such for the feeding experiment.

4.4. [3-^{14}C]N-Deacetylisoipecoside (**1**) and [3-^{14}C]N-deacetylipecoside (**2**)

Secologanin was condensed with [1-^{14}C]dopamine (pH 5) and the mixture was purified and resolved according to the reported procedure (Battersby et al., 1969b; Nagakura et al., 1978) to furnish [3-^{14}C]*N*-deacetylisoipecoside (**1**) and [3-^{14}C]*N*-deacetylipecoside (**2**).

4.5. [Ary-^{3}H]nordeoxytubulosine (**16**) and [aryl-^{3}H]deoxytubulosine (**17**)

Deoxytubulosine (**17**) was demethylated by HBr in AcOH to give nordeoxytubulosine (**16**). Acid-catalysed exchange reaction (Bhakuni et al., 1977, 1979) was employed to prepare [aryl-^{3}H]nordeoxytubulosine (**16**) and [aryl-^{3}H]deoxytubulosine (**17**).

4.6. Isolation of secologanin

Fresh leaves of *Lonicera japonica* (700 g) were percolated with EtOH (5×800 ml). The percolate was concentrated below 40 °C under reduced pressure to a thick syrup (40 g). The syrup was then diluted with H_2O (50 ml), defatted with benzene (6×50 ml) and extracted with *n*-BuOH (5×100 ml). The combined *n*-BuOH extract was washed with H_2O, dried (anhydrous Na_2SO_4) and concentrated in vacuo. The residue (20 g), so obtained, was chromatographed over silica gel (250 g) and eluted with $CHCl_3$ and $CHCl_3$:MeOH with increasing proportion of MeOH. Elution was monitored by TLC (plates: SiO_2GF_{254}; solvent: $CHCl_3$–MeOH; 8:2). Elution with $CHCl_3$–MeOH (9:1) gave secologanin (700 mg) as an amorphous powder $[\alpha]_D$ −95.6° (C, 1.12 in MeOH) (Budzikiewicz et al., 1964, $[\alpha]_D$ −96° MeOH).

4.7. Feeding experiment

For feeding purposes, dopamine, *N*-deacetylisoipecoside, *N*-deacetylipecoside, strictosidine and vincoside were dissolved in H_2O (1 ml). Tryptamine, nordeoxytubulosine and deoxytubulosine were dissolved in H_2O (1 ml) containing DMSO (0.2 ml). Labelled precursors were fed to young *A. lamarckii* Thw. plant by a wick feeding technique and when uptake was complete, H_2O was added for washing the precursor. The plants were then kept alive for 7–8 days to metabolise the precursor and then worked up for the biosynthetic alkaloids ankorine (**6**), deoxytubulosine (**17**) and alangimarckine (**20**).

4.8. Isolation of biosynthetic ankorine (**6**), deoxytubulosine (**17**) and alangimarckine (**20**)

Precursor fed young plants of *A. lamarckii* Thw. (180 g wet wt) were macerated in EtOH (300 ml) with inactive deoxytubulosine (100 mg), alangimarckine (80 mg) and ankorine (80 mg) and left for 20 h. After decanting the ethanolic solution, the marc was percolated with EtOH (5×250 ml). The combined ethanolic extract was concentrated in vacuo to afford a greenish viscous mass

which was then extracted with 10% AcOH (5×15 ml). The acidic extract was defatted with petroleum (4×20 ml), basified with Na_2CO_3 (pH 8–9) and the liberated bases were extracted with $CHCl_3$ (5×50 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (anhydrous Na_2SO_4) and solvent removed under reduced pressure. The crude mixture so obtained was subjected to preparative TLC (plates: thickness 0.3 mm; SiO_2GF_{254} solvent: $CHCl_3$–MeOH; 92:8; double run). The bands containing the desired alkaloids were cut-off and eluted with $CHCl_3$–MeOH (3:1), and the solvent removed in vacuo to give a series of residues. Pure biosynthetic deoxytubulosine (**17**) was crystallised from MeOH–Me_2CO, mp 230 °C (Battersby et al., 1965, 230–232 °C), alangimarckine (**20**), mp 174–175 °C (MeOH–Me_2CO) (Battersby et al., 1966, 174–6 °C) and ankorine (**6**), mp 173 °C (MeOH–Me_2CO) (DasGupta, 1965, 174 °C).

4.9. Degradation of the biosynthetic deoxytubulosine derived from [3-^{14}C]N-deacetylisoipecoside

A mixture of radioactive deoxytubulosine (**17**) (300 mg) (molar activity 8.44×10^5 dpm/mmol), pyridine (5 ml) and Ac_2O (5 ml) was heated on a water bath for 2 h, and then left at room temp. to furnish *N*-acetyldeoxytubulosine (**21**) (290 mg), mp 159–160 °C (MeOH–Et_2O) (Battersby et al., 1965, 158–160 °C) (molar activity 8.40×10^5 dpm/mmol). A solution of the preceding *N*-acetyldeoxytubulosine (**21**) in MeOH (5 ml) was refluxed with MeI (4 ml) for 2 h on a water bath and then left at room temp. for 20 h. Solvent from the reaction mixture was removed under reduced pressure and residue crystallised from MeOH to afford pure *N*-acetyldeoxytubulosine methiodide (**23**) (250 mg), mp 273–276 °C (molar activity 8.38×10^5 dpm/mmol); IR υ_{max}^{KBr}: 3420 (NH indole), 1625 ($NCOCH_3$), 1520, 1230 (OMe) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 0.85 (*m*, 3H, $CH_2C\underline{H}_3$), 1.2 (*m*, 2H, $C\underline{H}_2CH_3$), 2.45 (*s*, 3H, $NCOC\underline{H}_3$), 3.08 (*s*, 3H, $N^+C\underline{H}_3$), 3.71 and 3.72 (each *s*, 3H, each $2\times OC\underline{H}_3$), 5.65 (*m*, 1H, $\underline{H}_1$), 6.52 and 6.81 (each *s*, 1H each, $\underline{H}$-8 and $\underline{H}$-11) and 7.42–8.82 (*m*, 4H, Ar$\underline{H}$ 5′, 6′, 7′ and 8′), *m*/*z* 516 (M^+), 501 (M^+-15), 459 (M^+-57), 288 (M^+-228), 272–275 (M^+-244 to 241), 246 (M^+-270), 244 (M^+-272), 206 (M^+-310), 205 (M^+-311), 192 (M^+-324), 191 (M^+-325), 185 (M^+-331), 171 (base peak) (M^+-345) and 169 (M^+-347); (Found: C, 74.51; H, 7.98; N, 8.14. $C_{32}H_{41}O_3N_3$ requires C, 74.53; H, 8.01; N, 8.15%). A solution of the foregoing radioactive **23** (220 mg) in MeOH (100 ml) was passed through a column of freshly generated amberlite IR-410 anion exchange resin (OH^- form) (5 g) and the eluate recycled five times. The resin was finally washed with MeOH (100 ml). Solvent from the combined eluate was removed in vacuo to afford radioactive methohydroxide (**24**). **24** In MeOH (10 ml) was refluxed with KOH (3.5 g) in H_2O (1 ml) for 5 h, solvent from the reaction mixture was removed, H_2O (4.5 ml) added and then extracted with $CHCl_3$ (5×60 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (anhydrous Na_2SO_4) and solvent removed under reduced pressure to furnish radioactive *N*-acetyldeoxytubulosine methylmethine (**27**) (145.2 mg), crystallised from MeOH–C_6H_6, mp 173 °C (molar activity 8.30×10^5 dpm/mmol); IR υ_{max}^{KBr}: 3400 (NH indole), 2950, 1630 ($NCOCH_3$), 510 and 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.85 (*m*, 3H, $CH_2C\underline{H}_3$), 1.2 (*m*, 2H, CH_2CH_3), 1.95 (*s*, 3H, $NCOC\underline{H}_3$), 2.16 (*s*, 3H, $NC\underline{H}_3$), 3.76 and 3.84 (each *s*, 3H, each $2\times OC\underline{H}_3$), 5.0–5.52 (*m*, 3H, $C\underline{H}=C\underline{H}_2$), 6.72–7.4 (*m*, 5H, Ar$\underline{H}$), *m*/*z* 515 ($M^+$), 473 ($M^+$-42), 302 ($M^+$-213), 287 ($M^+$-228), 272 ($M^+$-243), 247 ($M^+$-268), 245 ($M^+$-270), 204 ($M^+$-311), 185 ($M^+$-330), 171 (base peak) ($M^+$-344) and 169 ($M^+$-346); (Found: C, 74.54; H, 7.99; N, 8.14. $C_{32}H_{41}O_3N_3$ requires C, 74.52; H, 8.01; N, 8.15%). Ozonised O_2 was passed through a solution of the radioactive **27** (132 mg) in EtOAc at −75 °C for 30 min, when the solvent from the resulting mixture was removed under reduced pressure. To the residue, H_2O (25 ml), Zn dust (250 mg) and $AgNO_3$ (20 mg) were added and refluxed for 20 min, then subjected to distillation. The distillate was collected in a solution of dimedone (300 mg) in aq. EtOH (3:1, 80 ml). The mixture was purified over silica gel column. Pure formaldehyde-dimethone (15 mg) was crystallised from MeOH–Et_2O, mp 188–189 °C (Battersby and Harper, 1962, 188 °C) (molar activity 8.20×10^5 dpm/mmol) (87% of original activity).

4.10. Degradation of the biosynthetic alangimarckine derived from [3-^{14}C]N-deacetylisoipecoside

Labelled alangimarckine (**20**) (62.8 mg) was diluted with inactive alangimarckine (252 mg), crystallised (molar activity 3.2×10^6 dpm/mmol) and treated with Ac_2O (5 ml) and pyridine (5 ml) to furnish *N,O*-diacetylalangimarckine (**22**) (300 mg) mp 144–145 °C (Battersby et al., 1969a, 143–146 °C) (molar activity 3.0×10^6 dpm/mmol). A solution of the radioactive **22** in MeOH (2 ml) was treated with MeONa (prepared from 50 mg Na in 1 ml MeOH) and MeI (5 ml). After 6 h, MeONa (1 ml) and MeI (2 ml) were added and after a total of 9 h solvent from the resulting mixture was removed under reduced pressure, then hot H_2O was added to the residue and filtered while hot. The filtrate was cooled and concentrated to give radioactive *N*-acetyl-*O*-methylalangimarckine methiodide (**25**) (280 mg), mp 199–206 °C (MeOH–H_2O) (molar activity 2.9×10^6 dpm/mmol); IR υ_{max}^{KBr}: 3400 (indole NH), 1625 ($NCOCH_3$), 1430 and 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 0.8 (*m*, 3H, $CH_2C\underline{H}_3$), 1.0 (*m*, 2H, $C\underline{H}_2CH_3$), 2.24 (*s*, 3H, $NCOC\underline{H}_3$), 3.29 (*s*, 3H, $N^+C\underline{H}_3$), 3.64, 3.76 and 3.85 (each *s*, 3H each, $3\times OC\underline{H}_3$), 5.74 (*m*, 1H, H-1′), 6.45 (*s*, 1H, $\underline{H}$-11) and 6.8–7.5 (*m*, 4H, Ar$\underline{H}$-5′,6′, 7′ and 8′), *m*/*z* 546 (M^+), 531(M^+-15),

489 (M^+-57), 318 (M^+-228), 302–305 (M^+-244 to 241), 276 (M^+-270), 274 (M^+-272), 236 (M^+-310), 235 (M^+-311), 222 (M^+-324), 221 (M^+-325), 185 (M^+-361), 171 (base peak) (M^+-375) and 169 (M^+-377); (Found: C, 72.61; H, 7.93; N, 7.71. $C_{33}H_{43}O_4N_3$ requires C, 72.62; H, 7.95; N, 7.70%). Methiodide **25** was converted into methohydroxide (**26**) as described earlier. Hofmann degradation of **26** as usual afforded *N*-acetyl-*O*-methylalangimarckine methylmethine (**28**) (180 mg), mp 198 °C (MeOH–C_6H_6) (molar activity 2.85×10^6 dpm/mmol); IR υ_{max}^{KBr}: 3400 (NH), 1630 ($NCOCH_3$), 1510 and 1450 (OMe) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (*m*, 3H, $CH_2C\underline{H}_3$), 1.18 (*m*, 2H, $C\underline{H}_2CH_3$), 2.11 (*s*, 3H, $NCOC\underline{H}_3$), 2.18 (*s*, 3H, $NC\underline{H}_3$), 3.73, 3.81 and 3.91 (each *s*, 3H, each $3\times OC\underline{H}_3$), 5.2–5.78 (*m*, 3H, $C\underline{H}=C\underline{H}_2$) and 6.99–7.5 (*m*, 5H, Ar$\underline{H}$); *m*/*z* 545 (M^+), 503 (M^+-42), 332 (M^+-213), 317 (M^+- 228), 302 (M^+-243), 277 (M^+-268), 275 (M^+-270), 234 (M^+-311), 222 (M^+-323), 221 (M^+-324), 185 (M^+-360), 171 (base peak) (M^+-374) and 169 (M^+-376); (Found: C, 72.63; H, 7.94; N, 7.69. $C_{33}H_{43}O_4N_3$ requires C, 72.62; H, 7.95; N, 7.70%). Ozonolysis of the foregoing methine in the usual manner afforded formaldehyde-dimethone (molar activity 2.6×10^6 dpm/mmol) (82% of original activity).

4.11. Degradation of the biosynthetic ankorine derived from [3-^{14}C]N-deacetylisoipecoside

Radioactive ankorine (**6**) (48 mg) was diluted with inactive ankorine (250 mg), crystallised (molar activity 3.65×10^4 dpm/mmol) and treated with an excess of ethereal CH_2N_2 to afford *O*-methylankorine (Bhakuni et al., 1979) (**7**) (280 mg) (molar activity 3.58×10^4 dpm/mmol). **7** In MeOH (5 ml) was refluxed with MeI (4 ml) for 4 h, and then left at room temp. to furnish *O*-methylankorine methiodide (**8**), mp 240 °C (dec.) (molar activity 3.50×10^4 dpm/mmol); *m*/*z* 349 (M^+-MeI), 348, 334, 318, 276, 235, 221, 220, 206, 164 and 163; (Found: C, 69.17; H, 9.43; N, 3.83. $C_{21}H_{34}O_4N$ requires C, 69.16; H, 9.41; N, 3.84%). The preceding methiodide was converted into methohydroxide (**9**) as described earlier. Hofmann degradation of **9** in the usual way afforded *O*-methylankorine methylmethine (**10**) (140 mg), mp 120 °C (MeOH–C_6H_6) (molar activity 3.45×10^4 dpm/mmol); IR υ_{max}^{KBr}: 3400, 2900, 1595 and 1450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.8 (*m*, 3H, $CH_2C\underline{H}_3$), 1.2 (*m*, 2H, $C\underline{H}_2CH_3$), 1.92 (*s*, 3H, $NC\underline{H}_3$), 3.72 (*s*, 3H, $OC\underline{H}_3$), 3.78 (*s*, 6H, $2\times OC\underline{H}_3$), 5.1–5.65 (*m*, 3H, $C\underline{H}=C\underline{H}_2$) and 6.82 (*s*, 1H, Ar$\underline{H}$); *m*/*z* 363 ($M^+$), 362, 348, 333, 319, 318, 304, 290, 276, 236, 234, 220 and 205; (Found: C, 69.16; H, 9.40; N, 3.82. $C_{21}H_{34}O_4N$ requires C, 69.18; H, 9.41; N, 3.84%). Ozonolysis of **10** in the usual way afforded formaldehyde–dimethone (molar activity 3.30×10^4 dpm/mmol) (90% of original activity).

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