LATE STAGES IN THE BIOSYNTHESIS OF IPECAC ALKALOIDS - CEPHAELINE, EMETINE AND PSYCHOTRINE IN *ALANGIUM LAMARCKII* THW. (ALANGIACEAE)

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ABSTRACT: The bioconversion of tyrosine, dopamine, N- desacetylisoipecoside (**15**) and N- desacetylipecoside (**16**) into cephaeline (**2**), emetine (**1**), and psychotrine (**3**) into *Alangium lamarckii* Thw. (Alangiaceae) plant has been studied. Stereospecific incorporation of N- desacetylisoipecoside (**15**) into **1,2** and **3** has been demonstrated. Further it has been shown that reduction of $C_{1'} - C_{2'}$ takes place after O-methylation of psychotrine (**3**). Feeding results further showed that cephaeline was poorly metabolized in the plants to form psychotrine (**3**) thus demonstrating that dehydrogenation of $C_{1'} - C_{2'}$ does not take place. The efficient incorporation of cephaeline into emetine (**1**) further showed that O- methylation is the terminal step in the biosynthesis of emetine. Emetine (**1**) was poorly metabolized by the plants to form cephaeline (**2**) and psychotrine (**3**). The experiments thus demonstrated that dehydrogenation and O-demethylation of emetine (**1**) does not occur to give cephaeline (**2**) and psychotrine (**3**).

KEY WORDS: Emetine; *Alangium lamarckii* Thw; Cephaeline; Psychotrine; Cephaelis ipecacuanha.

INTRODUCTION

Emetine (**1**), the well known antiamoebic[1] alkaloid and its relatives such as cephaeline (**2**), and psychotrine (**3**) were isolated from the dried rhizomes and roots of Cephaelis ipecacuanha[2] (Rubiaceae) and *Alangium lamarckii* Thw. (Alangiaceae) plant[3]. Emetine and tubulosine are widely used to treat amoebic dysentery. Emetine also has been used to treat schistosomiasis, leishmaniasis, and malaria. Among the side effects are cardiotoxicity, muscle weakness, and gastrointestinal problems. The alkaloid has a profound reversible effect on DNA synthesis.

The chemistry of these alkaloids has been reviewed[2]. The structure and stereochemistry of cephaeline, emetine and psychotrine as shown in 2, 1 and 3 respectively is well established. Several synthesis of emetine [4-7], psychotrine[8] and cephaeline[2] are reported.

We have reported the biosynthesis of a number of isoquinoline derived alkaloids in intact plants[10-12]. We have also established the stereochemistry at the two asymmeteric centres of biphenylbisbenzylisoquinoline alkaloids as exemplified by tiliacorine, tiliacorinine and nortilacorinine[13,14]. The early stages of the biosynthesis of these alkaloids have been studied [24-32]. The bioconversion of these bases has not yet been studied. We report for the first time, the late stages in the biosynthesis of emetine, psychotrine and cephaeline.

BIOGENESIS

A number of hypothesis[15-18] were put forward for the biogenesis of emetine and its relatives. The biosynthesis of cephaeline and emetine come from two main biosynthesis pathways. The isoquinoline moieties present in these alkaloids could be derived from tyrosine[12] or DOPA (**4**). The main controversy remained about the origin of nine carbon unit known as C_2-unit.Sir Robert Robinson[20] suggested that a protoberberine (**8**) type intermediate having a catechol system system can undergo "Woodward fission" to give C_2-unit, which can subsequently be modified to give a protoemetine intermediate (**10**). Condensation of **10** with dopamine, derived from dopa or tyrosine can then give the key intermediate (**11**) from which cephaeline (**2**), emetine (**1**) and psychotrine (**3**) can be formed. The second pathway starts with the biosynthesis of secologanin (**14**) from geranyl diphosphate. Biosynthesis begins from the reaction between dopamine (**5**) and secologanin (**14**) forming N-deacetylisoipecoside **(15)** (S-form) and N-deacetylipecoside **(16)** (R-form). The S-form (with retention of configuration) and R-form with inversion of configuration) can then go through a Pictet-Spengler type reaction followed by a series of O-methylations and the removal of glucose, with O-methyltransferases and a glycosidase, to

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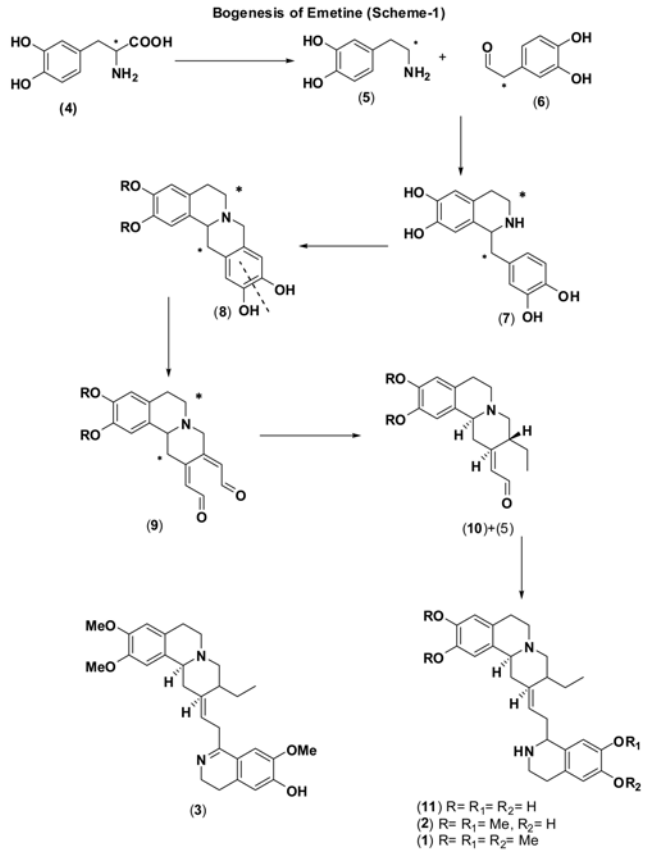


form proemetine. Proemetine then reacts with another dopamine molecule to form 7'-O-demethylcephaeline (**11**). The final products are then produced with a 7'-O-methylation to make cephaeline and a 6'-O-methylation successively to make emetine[21-26] (Scheme 1 and 2). The presence of ipecoside, an acetylated derivative of the first proposed intermediate lends credence to the proposed pathway.

RESULTS AND DISCUSSIONS

The results of various feeding experiments are recorded in Table1. Initial feeding of (L)-[U-^{14}C]-tyrosine (experiment 1) to young *Alangium lamarckii Thw*. (Alangiaceae) gave labeled emetine (**1**), cephaeline (**2**) and psychotrine (**3**) thus demonstrating that the enzyme system present in the plant is actively biosynthesising emetine (**1**), cephaeline (**2**) and psychotrine (**3**) (incorporation 0.077%, 0.26% and 0.015% respectively) at the time of feeding experiments.

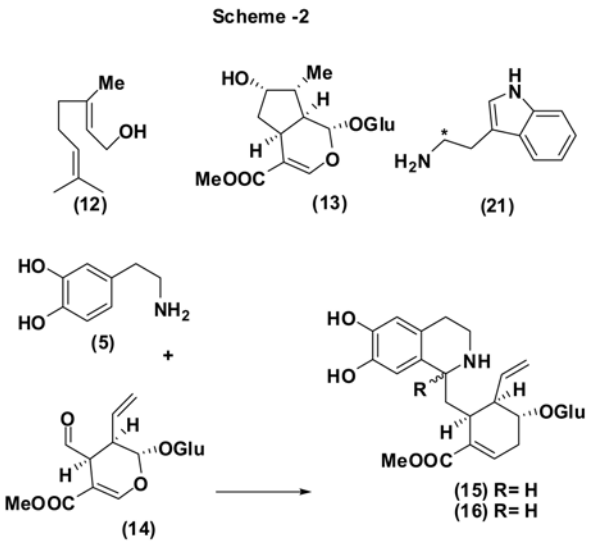
Feeding of [1-^{14}C]- dopamine (**5**) (experiment 1) demonstrated that dopamine was being efficiently utilized by the young *Alangium lamarckii* Thw. (Alangiaceae) plant to form emetine (**1**), cephaeline (**2**) and psychotrine (**3**).

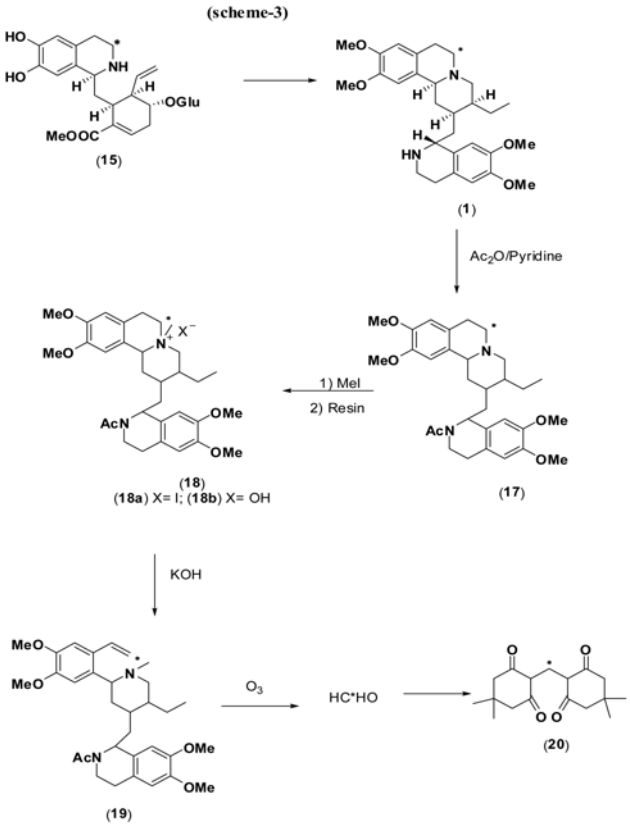
The epimeric monoterpenoid isoquinoline glucosides - N- Desacetylisoipecoside (**15**) and N- Desacetylipecoside (**16**) have á – and â – configuration at C_1 respectively. Cephaeline (**2**) emetine (**1**), and psychotrine (**3**) all have á – configuration at C_{11b} corresponding to C_1 position of the epimeric monoterpenoid isoquinoline glucoside. N-Desacetylisoipecoside (**15**), having á – configuration at C_1 can be converted into Cephaeline (**2**) emetine (**1**), and psychotrine (**3**) without any change in configuration at the corresponding position. If young *A. lamarckii* Thw. (Alangiaceae) plants chose N- Desacetylipecoside (**16**) have â – configuration at C_1 to form Cephaeline (**2**) emetine (**1**), and psychotrine (**3**), an inversion of configuration at C_1 must take place during the biotransformation of **16** into **2**, **1** and **3**.

Feeding of (L)-[U-^{14}C]-tyrosine in parallel with [3-^{14}C]-N- Desacetylisoipecoside (**15**) (experiment 3) and [3-^{14}C]-N- Desacetylipecoside (**16**) (experiment 4) to young *A. lamarckii* Thw. (Alangiaceae) plants demonstrated that the stereospecificity is maintained in the biosynthesis of cephaeline (**2**) emetine (**1**), and psychotrine (**3**). The former was incorporated about 23 times more efficiently than the later.

Regiospecificity of label in the biosynthetic emetine (**1**) derived from the feeding of with [3-^{14}C]-N-Desacetylisoipecoside (**15**) (experiment 3) was established as follows: Labeled emetine (**1**) was treated with acetic anhydride and pyridine to give radioactive N-acetylemetine (**17**) with essentially the same radioactivity as parent base. Radioactive **17** was converted into radioactive methiodide (**18a**) by refluxing with methyl iodide with no loss of radioactivity. Radioactive methiodide (**18a**) in MeOH was passed through amberlite IR-400 anion exchange resin (OH^- form) to give the corresponding radioactive methohydroxide (**18b**). **18b** Was refluxed with 35% potassium hydroxide solution to yield radioactive methine (**12**) with essentially the same molar radioactivity as parent base. Ozonolysis of 12 gave formaldehyde trapped as its dimedone derivative (**20**) having essentially 25% original radioactivity.

To study the biointerconversion of cephaeline (**2**) emetine (**1**), and psychotrine (**3**), labeled [aryl-^{3}H]- cephaeline (**2**) (experiment 5), [aryl-^{3}H]- emetine (**1**) (experiment 6), and [aryl-^{3}H]- psychotrine (**3**) (experiment 7) were fed in parallel to young *A. lamarckii* Thw. (Alangiaceae) plants. The feeding results showed that psychotrine (**3**) incorporated into both cephaeline (**2**) and emetine (**1**) more efficiently than cephaeline (**2**) thus demonstrating that reduction of $C_{1'} - C_{2'}$ takes place after O-methylation. Feeding results further showed that cephaeline was poorly metabolized in the plants





to form psychotrine (**3**) thus demonstrating that dehydrogenation of $C_{1'}-C_{2'}$ does not take place. The efficient incorporation of cephaeline into emetine (**1**) further showed that O- methylation is the terminal step in the biosynthesis of emetine. Emetine (**1**) was poorly metabolized by the plants to form cephaeline (**2**) and psychotrine (**3**). The experiments thus demonstrated that dehydrogenation and O-demethylation of emetine (**1**) does not occur to give cephaeline (**2**) and psychotrine (**3**).

CONCLUSION

The foregoing tracer experiments strongly support the following sequence for the biosynthesis of emetine in *Alangium lamarckii* Thw. Plant:

Tyrosine '! dopamine (**5**) + secologanin (**14**) '! N desacetylisoipecoside (**15**) '! psychotrine (**3**) '! cephaeline (**2**) '! emetine (**1**).

EXPERIMENTAL

Melting points were taken in sulphuric acid bath and are uncorrected. The ^{1}H NMR spectra were recorded on a Perkin Elmer P-30 (20 MHz) spectrometer. Mass spectra were recorded on a jeol JMS D 300 spectrometer at an ionization energy of 70eV. 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[10-14, 2, 28].

Isolation Of Emetine (1), Cephaeline(2) and psychotrine(3)

Air-dried root bark (5kg) of *Alangium lamarckii* Thw. (Alangiaceae) was pulverized and percolated with ethanol (4x3 lit). The percolate was concentrated under reduced pressure below 40° to afford the green viscous mass which was extracted with 5% AcOH (5 x 300 ml). The combined aqueous acidic solution was defatted with ether (5 x 100 ml), basified with Na_2CO_3 (pH 8) and the liberated bases extracted with $CHCl_3$ (7 x300 ml). The $CHCl_3$ extract was washed with H_2O, dried (anhydrous Na_2SO_4) and concentrated *in vacuo* to give a crude alkaloidal mixture (4.0 g) which was chromatographed over a column of neutral Al_2O_3 (150 g). Elution of the column with C_6H_6: EtOAc (1:1) (TLC control) gave Emetine (**1**) (0.5 g) as an amorphous powder, [á] $_D$ – 26.0° ($CHCl_3$) (Lit.[2], [á] $_D$ – 26.0°,$CHCl_3$). Continued elution with EtOAc: MeOH (25:5 and 85:15) gave cephaeline (**2**) (0.6g) crystallized from Et_2O, m.p. 104° , [á] $_D$ – 22.5° ($CHCl_3$) (Lit.[2] m.p. 104-107° [á] $_D$ – 23.4°,$CHCl_3$) and psychotrine (**3**) (0.55g), m.p. 102° , [á] $_D$ + 44.2° ($CHCl_3$) (Lit.[2] m.p. 108-112° [á] $_D$ + 48.0°, $CHCl_3$) respectively.

Isolation of Secologanin

Fresh leaves of *Lonicera japonica* (200g) were percolated with EtOH (5x500ml). The percolate was concentrated below 40° under reduced pressure to a thick syrup (12g). The syrup was diluted with H_2O (50ml) and defatted with benzene (6x 50ml) and extracted with n-butanol (5X100ml). The combined n-butanol extract was washed with H_2O, dried (anhy. Na_2SO_4) and concenterated in vaccum. The

Table 1: Tracer Experiments on *A. lamarckii* Thw. Plant.

Experiment	Precursor fed	Incorporation % into		
		Emetine (**1**)	Cephaeline (**2**)	Psychotrine (**3**)
1.	(L)- [U^{14}C] – Tyrosine	0.077	0.26	0.015
2.	[1-^{14}C] – Dopamine (**5**)	0.16	0.18	0.124
3.	[3-^{14}C]-N- Desacetylisoipecoside (**15**)	0.07	0.34	0.11
4.	[3-^{14}C]-N- Desacetylipecoside (**16**)	0.003	0.002	0.005
5.	[Aryl-^{3}H]- Cephaeline (**2**),	1.285	-	0.116
6.	[Aryl-^{3}H]- Emetine (**1**)	-	0.0114	0.0013
7.	[Aryl-^{3}H]- Psychotrine (**3**)	6.18	2.75	-

residue (5.2g), so obtained, was chromatographed over a column of silica gel (250g). The elution was effected with $CHCl_3$ and $CHCl_3$:MeOH with increasing proportion of MeOH. Elution was monitored by TLC (plate: SiO2 GF_{254}; solvent: $CHCl_3$: MeOH, 8:2). Fractions collected were each of 100ml. elution with $CHCl_3$:MeOH (2:1) gave secologanin (**14**) (212mg) as an amorphous mass, $[á]_D$- 25.6^0 (c, 1.12 MeOH) (lit.[22], $[á]_D$-26^0 MeOH).

Synthesis and Labelling of Precursors :

Isolation of secologanin[22], synthesis of [1-^{14}C]-Tryptamine hydrochloride[30,31], [1-^{14}C]-3,4 – Dihydroxyphenethylamine[32,33] (Dopamine) and [3-^{14}C]-N-Desacetylisoipecoside (**15**) and [3-^{14}C]-N-Desacetylipecoside[34] (**16**) were done by standard procedure[28].

[Aryl-^{3}H]- Cephaeline (**2**) - A mixture of Cephaeline (25mg), T_2O (0.2ml, activity = 200mCi) and $SOCl_2$ was heated in a sealed tube under nitrogen atmosphere at 100^0 for 20 hr. Work up as usual afforded [Aryl-^{3}H]- cephaeline (**2**).

[Aryl-^{3}H]- Psychotrine (3) was also prepared by the above procedure.

Feeding Experiments

For feeding purposes, labeled tyrosine, dopamine, N-desacetylisoipecoside (**15**) and N-desacetylipecoside (**16**) were dissolved in H_2O (1ml), labeled emetine (**1**), cephaeline(**2**) and psychotrine (**3**) were dissolved in H_2O (1ml) containing DMSO (0.2ml). Labeled precursors were fed to young *A. lamarckii* Thw. plants (3nos.) by a wick feeding technique and when uptake was complete water was added to wash the precursor. The plants were kept alive for 7-8 days to metabolize the precursor and then worked up for the biosynthetic alkaloid emetine (**1**), cephaeline (**2**) and psychotrine (**3**) respectively.

Isolation of Biosynthetic Emetine (1)

The precursor fed young plants of (60g wet wt) was macerated in EtOH (500ml) with inactive emetine (80mg) in EtOH(300 ml) and left for 20 hrs. The ethanolic solution was decanted and the marc was percolated with fresh EtOH (5 x 250 ml). The combined ethanolic extract was concentrated *in vacuo* to afford a green viscous mass which was then extracted with 5% AcOH. The acidic extract was defatted with petroleum ether (5 x 20ml), basified withNa_2CO_3 (pH 8) and the liberated bases were extracted with $CHCl_3$ (5 x 100 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (anhyd. Na_2SO_4) and solvent removed under reduced pressure to afford the crude base. The crude mixture so obtained was subjected to preparative thin layer chromatography (plate thickness 0.3 mm, SiO_2GF_{254}, solvent: $CHCl_3$:MeOH, 23:7). The band containing emetine (**1**), cephaeline (**2**) and psychotrine (**3**) respectively were cut-off and eluted with $CHCl_3$:MeOH and the solvent removed in vacuo to give radioactive emetine (60.8 mg), cephaeline (52.5mg) and psychotrine (50.2mg).

Isolation of labeled emetine, cephaeline and psychotrine was similarly done from the feeding of various labeled precursors.

Degradation Experiments

Degradation of labeled Emetine derived from [3-^{14}C]-N-Desacetylisoipecoside (15)

A solution of radioactive emetine (**1**) (303 mg) (molar activity 4.778 x 10^5 dis/min/mmol) derived from feeding of [3-^{14}C]-N- Desacetylisoipecoside (**15**) (experiment 3) was refluxed with pyridine (5ml) and acetic anhydride (5ml) for 1hr and then left overnight at room temperature. Excess of pyridine and acetic anhydride were removed from the resulting mixture and H_2O added to the residue. The product thus obtained was extracted with $CHCl_3$ extract, washed with H_2O, dried (anhyd. Na_2SO_4) and solvent removed under reduced pressure. The crude product was passed through a column of silica gel to afford pure N-acetyl emetine (**17**) (285mg), m.p. 24^0 (Lit.[35], m.p. 27-22^0) (molar activity 4.65 x 10^5 dis/min/mmol). The preceding radioactive N-acetyl emetine (**17**) (275mg) in MeOH (4 ml) was refluxed with methyl iodide (4 ml) for 4hr to give radioactive N-acetyl emetine methiodide (**18a**) (270 mg), m.p. 210^0 (Lit.[35], m.p. 213-16^0) (molar activity 4.68 x 10^5 dis/min/mmol).

A solution of the foregoing radioactive N-acetyl emetine methiodide (**18a**) (250 mg) in MeOH (100 ml) was passed through a column of freshly generated amberlite IR-400 anion exchange resin (OH^- form) (5.0 g) and the solution was recycled five times. The column was finally washed with MeOH (150 ml). Solvent from the combined eluate was removed to furnish radioactive base methohydroxide (**18b**). Labelled methohydroxide in aqueous MeOH (10 ml) was refluxed with KOH (4.4 g) in H_2O (2 ml) for 5hr on a water bath. Methanol from the resulting mixture was removed, H_2O (10 ml) added and the product extracted with $CHCl_3$ (5x50 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed *in vacuo* to furnish radioactive methine (**19**) (200 mg) (molar activity 4.63 x 10^5 dis/min/mmol).

Ozonised was passed through a solution of radioactive **19** (120mg) in EtOAc (15ml) at -78^0 for 30 min. The solvent from the resulting mixture was removed. To the residue H_2O (25ml), Zn dust (250mg) and $AgNO_3$ (20mg) were added. The resulting mixture was refluxed for 20 min. And then subjected to distillation. The distillate was collected in a solution of dimedone (300mg) in aqueous EtOH (3:1, 80ml) and left for 1hr at room temperature. It was then concentrated to 10ml and left overnight. The product, so obtained, was filtered, washed with H_2O, dried (anhyd. Na_2SO_4) and chromatographed on a column of silica gel. Elution with C_6H_6 and C_6H_6 : $CHCl_3$ gave formaldehyde dimethone (**20**) (25mg) m.p.187^0(Lit. [36],m.p. 188^0) (molar activity 4.65 x 10^5 dis/min/mmol) (25% of original radioactivity).

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