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BIOSYNTHESIS OF ALPINIGENINE BY WAY OF TETRAHYDROPROTOBERBERINE AND PROTOPINE INTERMEDIATES*

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Key Word Index—*Papaver bracteatum*; Papaveraceae; benzazepine alkaloid biosynthesis; alpinigenine; ring transformation by way of *N*-quaternization and subsequent hydroxylation at α-carbon.

Abstract—In the biosynthesis of the benzazepine alkaloid alpinigenine a *N*-methylation step followed by hydroxylation α to nitrogen has now been shown more conclusively to be involved in the transformation of a *N*-heterocyclic ring system. After feeding *Papaver bracteatum* plants both the precursors (±)-tetrahydropalmatine-[8,13,14-^{3}H] and (±)-tetrahydropalmatine methiodide-[8,13,14-^{3}H;8-^{14}C] an identical mode of abstraction of tritium was observed including a complete loss of the isotope from C-14. The next member in the biogenetic chain, muramine-[8-^{14}C], was incorporated into alpinigenine very efficiently. Furthermore, using structurally different precursors not utilized for normal alkaloid formation, e.g. 2′-hydroxymethyl-laudanosine-[$^{14}CH_2OH$], 13-hydroxymuramine-[8-^{14}C], the specificity of alkaloid metabolism was examined in the whole plant. Tracer dilution technique was applied to confirm the occurrence in the plant of three established intermediates. Chemical syntheses of four of the alkaloids used during these investigations were developed.

INTRODUCTION

In preliminary biosynthetic studies [1, 2] the tetrahydrobenzazepine alkaloid alpinigenine (**5**) [3, 4] representing the *trans* series of rhoeadine-type bases [5] has been shown to originate in *Papaver bracteatum* Lindl., the scarlet poppy, from two molecules of tyrosine-[3-^{14}C]. In addition six C_1-units were incorporated into the four *O*-Me, the *N*-Me and the hemiacetal carbon atom C-14 after feeding of methionine-[*S*-^{14}Me].

Subsequently, tetrahydropalmatine (THP) (**1**) could be established as a major precursor of alpinigenine (**5**) in the same plant [6]. THP or analogous compounds possessing a tetrahydroprotoberberine skeleton have been shown to be involved as key intermediates in the biosyntheses of various isoquinoline derived alkaloids, i.e. the protopine, benzophenanthridine, and phthalide-isoquinoline types, respectively [7]. From this it can be assumed that in *P. bracteatum* too THP is formed from tyrosine by way of norlaudanosoline, (+)-reticuline, and (−)-scoulerine (Scheme 1). Concerning reticuline there are observations which show that the (+)-isomer is only a precursor of **5** whereas the (−)-enantiomer is used in the biosynthesis of thebaine [8]. In the above-mentioned paper [6] the *N*-quaternary derivative **2** was suggested as the next member in the biogenetic sequence and a novel ring opening mechanism was proposed which consists in α-hydroxylation of a *N*-quaternary compound like **2**. Unfortunately, in all the feeding experiments with **2** the incorporation of radioactivity was markedly lower than with its predecessor **1**. This might be due to a limited uptake of the quaternary precursor **2** by the plant, or different transport mechanisms which lead to different concentrations of **1** and **2**, respectively, at the site of alkaloid synthesis, or eventually competition with hitherto unknown pathways transforming the major amount of **2** into undetected metabolites. Also it has not been possible to exclude that labelled **2** is rearranged into **5** in an unphysiological way. Further evidence confirming the actual role of *N*-methylation of a tetrahydroprotoberberine intermediate in the biosynthesis of **5** has now been gained from the experiments described here.

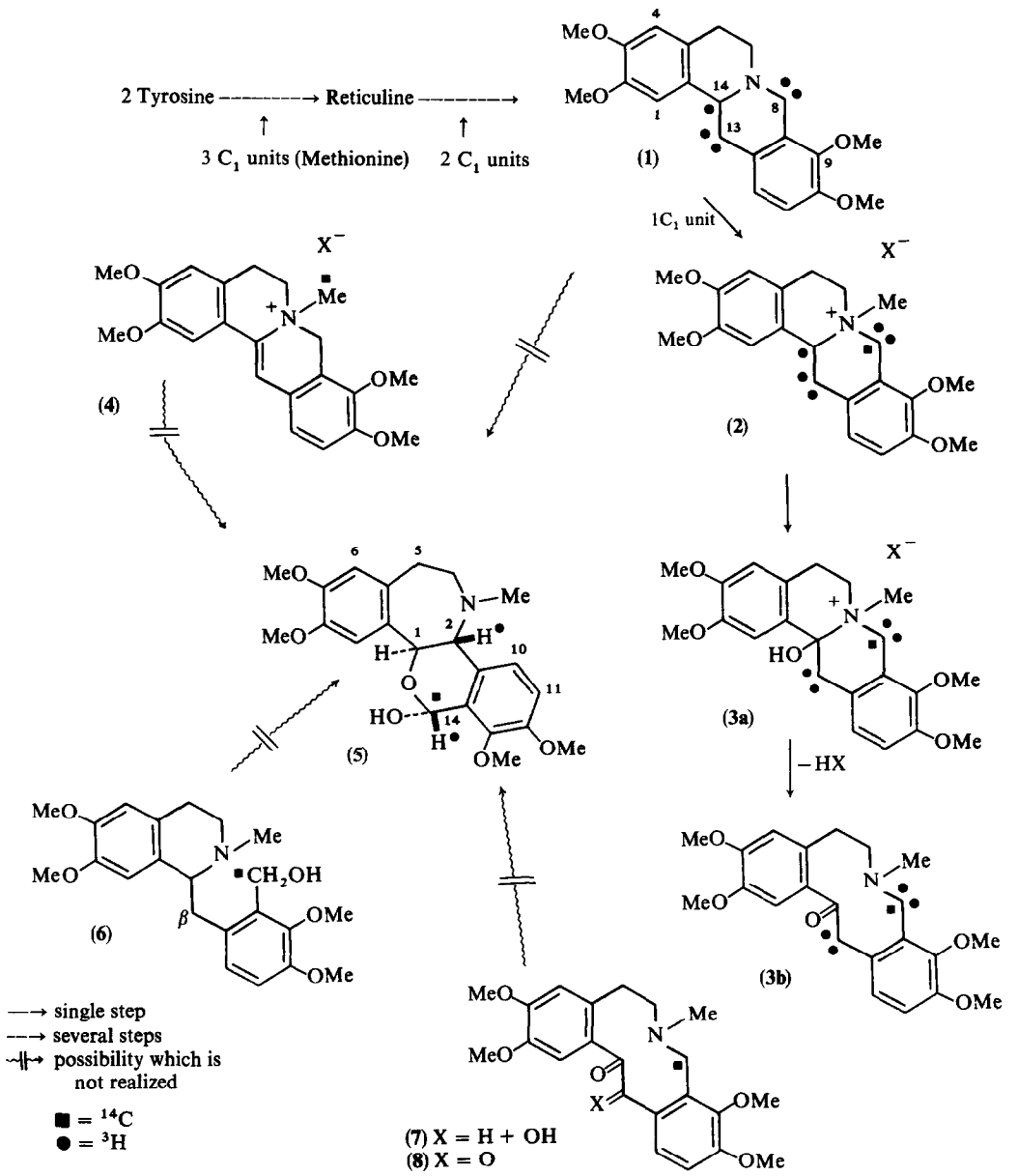
RESULTS AND DISCUSSION

Table 1 comprises the results obtained with radioactively labelled substances which have been fed by a method described earlier [1, 6] to flowering scarlet poppy plants grown in the open air. Following the usual isolation procedure, the alkaloids alpinigenine (**5**) and the morphinane base thebaine have been obtained from the aboveground plant material harvested after a 20-day period of incubation with the precursors. To disclose the distribution among specific positions of labelled atoms (^{3}H and/or ^{14}C) a previously developed degradation procedure [7] has been applied to significantly active **5**, which was crystallized to constant radioactivity. Thebaine on the other hand, which is formed by a partly-independent pathway [8] not involving THP, was never found to incorporate radioactivity from the alpinigenine-specific precursors listed in Table 1.

Synthesis of THP-[8,13,14-^{3}H] and feeding experiment 1

The enzyme mediated rearrangement of a tetrahydroprotoberberine ring system into a 2-phenyltetrahydro-3H-3-benzazepine skeleton encountered in **5** ought to involve chemical reactions at carbons 8, 13 and 14 and

* Contribution No. XI in the series 'On *Papaver bracteatum*'; No. X cf. Rönsch, H., (1972) *Tetrahedron Letters*, 4431.



Scheme 1. Biosynthesis of alpinigenine (5).

Table 1. Feeding experiments on *Papaver bracteatum* plants

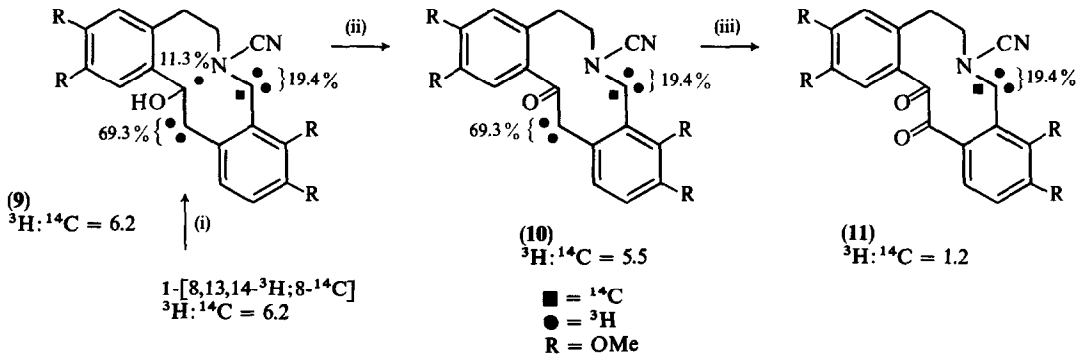
No. of experiment	Precursor	Kind and position of label	Sp. act. (dpm/mM)	Amount fed (mg)	Isolated alpinigenine (5) (dpm/mM)	(mg)	(% incorp.)
1	(±)-THP-methiodide (2)	8,13,14-^{3}H;8-^{14}C	2.62×10^{8}*	45.0	5.20×10^{5}*	195	1.0
2	(±)-THP (1)	8,13,14-^{3}H	3.49×10^{9}	41.5	5.19×10^{7}	180	10.4†
3	Muramine (3b)	8-^{14}C	1.31×10^{9}	34.1	3.00×10^{7}	350	22.6
4	Dihydropalmatine-methosulphate (4)	*N*-^{14}Me;[SO_4 ^{14}Me]$^-$	4.72×10^{8}	46.3	9.90×10^{4}	197	0.1
5	(±)-2′-Hydroxymethyl-laudanosine (6)	$^{14}CH_2OH$	1.93×10^{9}	24.3	8.80×10^{3}	336	—
6	(±)-13-Hydroxy-muramine (7)	*N*-^{14}Me	4.72×10^{8}	41.0	2.44×10^{3}	114	—
7	13-Oxo-muramine (8)	*N*-^{14}Me	4.72×10^{8}	11.5	2.40×10^{3}	64	—

* ^{14}C-activity. † Calculated in allowance for a ^{3}H-loss amounting to 55.6% of the original THP radioactivity, which is due to the elimination of the one hydrogen atom from C-8, C-13, and C-14 each (cf. Schemes 2 and 3).

also at the nitrogen atom. To obtain greater insight into this process both precursors, THP and THP-methiodide, were labelled with ^3H at these key centres whereas ^{14}C served as an internal standard in feeding experiment 1 described in the sequel (for experiment 2, see later sections).

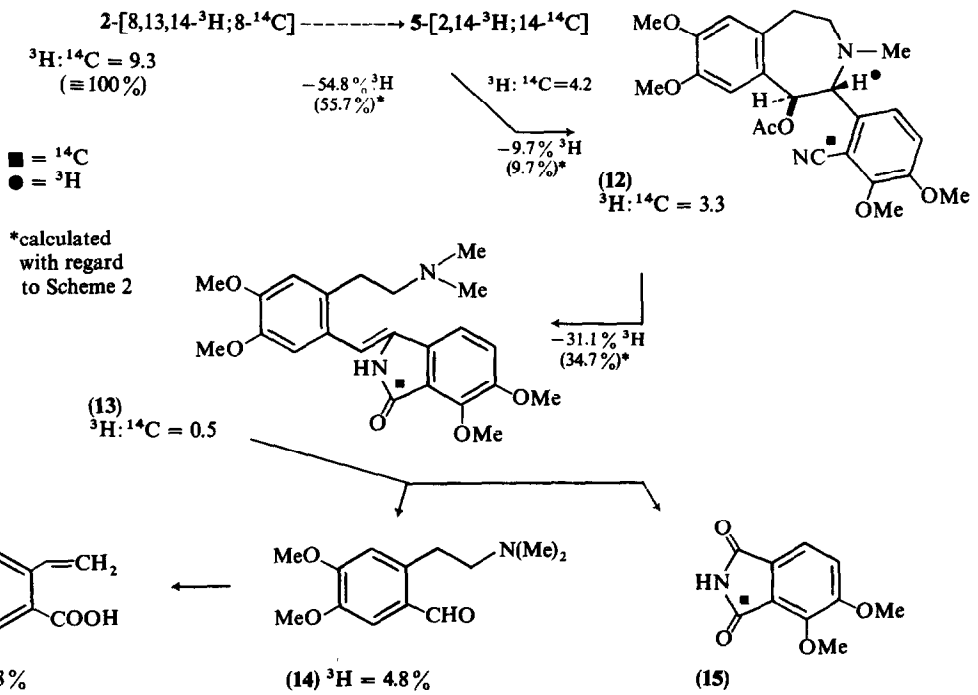
The preparation of THP-[8,13,14-^3H;8-^{14}C] from palmatine chloride-[8-^{14}C] was achieved with $NaBH_4$-[^3H] in dimethyl sulphoxide; the addition of a small amount of tritiated water to the carefully dried solvent proved to be essential for the introduction of label at C-13. In ethanol without tritiated water, ^3H could be introduced only into positions 8 and 14. Apparently, the $\Delta^{N.8}$ double bond is the first one to be hydrogenated and subsequently the other is translocated into the $\Delta^{N.14}$ position, a proton or $^3H^+$ from the solvent being added to C-13 simultaneously. In THP synthesized in the presence of tritiated water, consequently, rather different amounts of ^3H were encountered at C-8, C-13 and C-14, respectively. A simple degradation of THP (Scheme 2) showed the distribution of label among these positions. Another tetrahydroprotoberberine, stylopine, has been reported to be labelled at C-8 in a rather similar way using borotritiide in anhydrous dimethylformamide but no degradation was carried out [10].

In feeding experiment 1 an aqueous solution of (±)-THP-methiodide-[8,13,14-^3H;8-^{14}C] was administered to *P. bracteatum*. 2 exists in two stereoisomeric forms, α and β, each of which might be the true precursor. Therefore 2 was used as a crude mixture formed from THP and methyl iodide in which the α-isomer (*cis*) is the minor constituent, which would have been lost on crystallization. The incorporation of ^{14}C label into alpinigenine (5) was greater than in the previous experiments [6]. Moreover, the presence of an internal radioactive standard showed a loss of 54.8% of the tritium in the precursor. This value accounts very well for the loss of either hydrogen from C-8, C-13, and additionally,



(i) $BrCN-H_2O-MgO-THF$. (ii) CrO_3–Py. (iii) SeO_2–dioxane.

Scheme 2. Degradation of multiply labelled 1.



Scheme 3. Degradation of 5 from feeding experiment 1.

one from C-14. Consequently, if no intra- or intermolecular hydrogen transfer occurs, the other labelled hydrogens should be found in alpinigenine at C-2 and C-14. In order to confirm this, a chemical degradation of multiply labelled **5** was performed. Each of the biogenetically important hydrogen atoms was eliminated in a separate step from the sites in question. Since the related chemical work had been dealt with previously [5a, 9] the results obtained by the degradation of ^{3}H-labelled **5** are shown in Scheme 3.

Degradation of alpinigenine (**5**) *from feeding experiment 1*

In the first step the C-14 hydrogen originating from C-8 in THP was abstracted and an equivalent decrease of the ^{3}H:^{14}C ratio was determined in the nitrile derivative **12**. Obviously one of the two C-8 hydrogen atoms had been retained at C-14 in biosynthesis of **5**. This result is at variance with an earlier biogenetic proposal [5b] involving a phthalideisoquinoline intermediate, which would implicate the total loss of tritium from C-8 (cf. also experiment 5).

The benzylidenephthalimidine derivative **13**, which was formed from the foregoing nitrile, appeared to contain no more tritium. The label thus eliminated from C-2 was approximately as expected for the loss of a labelled hydrogen stemming from the THP-C-13. This result clearly indicates that two further hydrogen atoms were lost in the biotransformation of THP leading to **5**, namely a second one from C-13 and a single one from C-14. It can be concluded that compounds like **3b** or **4** lacking hydrogen at C-14 must be potential intermediates.

However, by degradation **13** was shown to contain a small but appreciable amount of tritium which had been obscured due to both, apparently, the ^{14}C-label still present and the yellow colour of the compound causing quenching in liquid scintillation counting. Thus, a Lemieux Johnson oxidation of **13** afforded ^{14}C-labelled hemipinimide (**15**) in addition to the veratraldehyde derivative **14**, which still showed unexpected tritium amounting to 4.8% rma (= relative molar activity). By degradation to the acid **16**, the major part of this activity was detected at the aldehydic centre, i.e. C-1 which corresponds to THP-C-14. The observed difference of 3.0% rma attributable to hydrogen at C-1 seems to be too large to be neglected but otherwise too small (25% of the original label at C-14) to be rationalized in terms of an isotope effect operating with partial retention of tritium. A simple explanation of this result would be that reincorporation of label previously eliminated from THP-C-8 or THP-C-13 takes place at 5-C-1 to a considerable degree. This would require the sequence of dehydrogenation and hydrogenation at this carbon atom occurring inside the same cell compartment or on a multi enzyme complex.

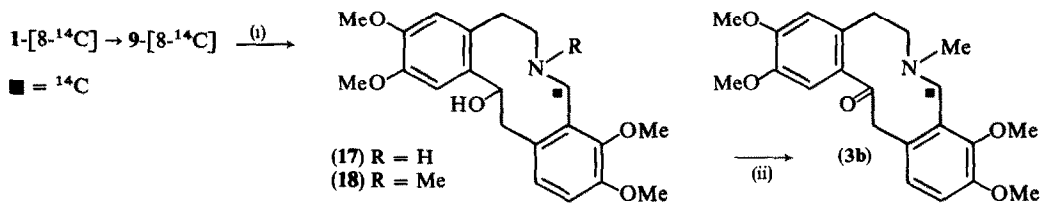
Protopine-type precursor **3b** *and novel ring-opening mechanism*

In searching for the precursor in the biogenetic chain before **2** which apparently should be devoid of hydrogen at C-14, a feeding experiment with the 14-ketone muramine-[8-^{14}C] (**3b**) was performed. This protopine-type alkaloid has been synthesized [11] and later isolated from *Papaver nudicaule* var. *amurense* [12]. A new and efficient synthesis of this possible precursor from THP-[8-^{14}C] prepared as indicated earlier [6] was developed [13] because low and/or extremely variable yields had been encountered with the syntheses along known routes [14] of these alkaloids. Being formed in a high yield from THP-[8-^{14}C] by solvolytic BrCN reaction in a buffered medium [15] the 14-hydroxy-cyanamide (**9**) was a suitable starting compound. **9** was hydrogenolyzed using $LiAlH_4$ to give the related secondary amine **17** which in turn was methylated (CH_2O–$NaBH_4$). The resulting 14-dihydromuramine (**18**) could be dehydrogenated smoothly with pyridinium chlorochromate in methylene chloride [16] (Scheme 4). The labelled protopine **3b** now accessible in a little over 35% over-all yield, was administered to the plant in the usual way (Table 1, experiment 3). The precursor was efficiently converted into alpinigenine (**5**), more than 20% of the total amount of radioactivity being incorporated. Using a degradation procedure similar to Scheme 3, the label was shown to reside at C-14 (Table 2). The results obtained indicate a novel kind of pathway in alkaloid biogenesis consisting in an *N*-methylation step which is followed by hydroxylation at C-14, the carbon atom adjacent to the quaternary nitrogen of the tetrahydroprotoberberine **2**. A non-enzymatic step seems to complete the fission of the *N*-C-14 bond (cf. Scheme 1). Protopine-type alkaloids have long been known to exist in

Table 2. Degradation of labelled **5** from expt 3

	dpm/mM	rma*
Alpinigenine (**5**) (diluted)	1.04×10^7	100
Nitrile derivative **12**	1.01×10^7	98
Phthalimidine derivative **13**	1.03×10^7	99
Methiodide of veratraldehyde **14**	$<0.02 \times 10^7$	<2
Hemipinimide (**15**)	1.07×10^7	103
Hemipinimide (diluted)	1.59×10^5	100
5,6-Dimethoxyanthranilic acid	1.61×10^5	101
3,4-Dimethoxyanthranilic acid	0.03×10^5	2

* Relative molar activity.



(i) (**17**): $LiAlH_4$; (**18**): CH_2O–$NaBH_4$. (ii) $C_5H_6N^+[CrO_3Cl]^-$.

Scheme 4. Synthesis of labelled muramine (**3b**).

two forms [5b], for example **3a** and **3b**, which can be transformed into each other easily by a change of pH. Formula **3a** represents the cation of the salts with acids.

In alkaloid chemistry nucleophilic attack seems to be rather uncommon at an α-carbon atom bound to a normal quaternary nitrogen; analogously, in alkaloid biosynthesis an involvement of *N*-quaternary compounds has rarely been considered. Biogenetic theories [18] have stressed the importance of Schiff's bases which in several cases have been found to be intermediary in the rearrangement of nitrogenous skeletons into other, more complex ones. Very recently a quaternary dihydroprotoberberine derivative corresponding to **4** was reported to play a key role in the biosynthesis of the benzophenanthridine alkaloid chelidonine in *Chelidonium majus* [10]. In benzazepine alkaloid biosynthesis however a different mechanism seems to operate with a different quaternary intermediate. Accordingly, if fed to *P. bracteatum* (experiment 4) the labelled enamine methosulphate **4** did not give specific incorporation into alpinigenine (**5**) and by degradation (Table 3) the low activity observed in **5** was shown to be scattered over the methoxy groups. In current work on alkaloid biosynthesis, another example of this has been obtained. Protopine has been shown to be formed from stylopine, a bismethylenedioxytetrahydroprotoberberine, by way of the corresponding methosalt not only in *Chelidonium majus* [10] but also in *Corydalis incisa* [17]. Moreover, in these experiments there was no conversion of the saturated quaternary precursor into a benzophenanthridine formed by the same plant, namely chelidonine or corynoline, respectively.

Further evidence in favour of the novel mechanism proven by experiments 1, 3 and 4 comes from experiment 2. The aim in feeding THP-[8,13,14-^{3}H] (**1**) was to make a comparison with the multiply labelled quaternary precursor **2** (experiment 1). Upon degradation (Table 4), the alpinigenine (**5**) obtained exhibited the same labelling features as observed in experiment 1. Obviously, both the precursors are involved into the same pathway. It will be noted that practically all the tritium residing at THP-C-14 was eliminated; this was the only difference between the two experiments.

Table 3. Degradation of **5** from expt 4

	dpm/mM ($\times 10^4$)	rma
Alpinigenine (**5**)	8.5	100
Nitrile derivative **12**	8.6	101
Phthalimidine derivative **13**	8.0	94
Phthalimidine after dilution	2.7	100
Hemipinimide (**15**)	0.94	35
Veratraldehyde derivative **14**	1.8	67

The problem of precursor specificity

In recent years, non-physiological substrates have been fed to plants and converted to aberrant products not normally present [19]. As mentioned above, the possibility has to be taken into consideration that the incorporation of the quaternary precursor **2** into **5** might be due to such an unphysiological interaction with plant enzymes. With this idea in mind, a series of three experiments were carried out to determine the degree of specificity in alkaloid biosynthesis within *P. bracteatum* (cf. Table 1, feedings 5, 6, 7). In the first of these experiments, the possibility was examined, that the THP-*N*-C-8 bond could be the first of the two nitrogen bonds to be cleaved on the route to **5**. The hitherto unknown 2'-hydroxymethyl-laudanosine (**6**) was selected for feeding because it constitutes a suitable 7,8-seco-tetrahydroprotoberberine; **6** corresponds to a phthalideisoquinoline-type with respect to both fundamental structure and oxygen function at the C_1-unit bound to C-2'. These lactonic isoquinolines have been suggested to be intermediary in rhoeadine alkaloid biosynthesis [5a, 5b, 20, 21].

The synthesis of **6** was performed smoothly using a recent variety of the long known ethyl chloroformate degradation. Thus, THP known to be inert against ethyl chloroformate [22] was now attacked at the *N*-C-8 bond by this reagent in the presence of NaI [23] in acetone to form the corresponding 2'-iodomethylene compound **19**. The iodine of the latter was subsequently exchanged using sodium acetate and the product was reacted with $LiAlH_4$ to give the 2'-hydroxymethyl-benzyl-tetrahydroisoquinoline **6**.

The labelled compound **6** synthesized in a similar way from THP-[8-^{14}C] was absorbed by the plant after administration; as expected it resulted in alpinigenine (**5**) carrying negligible radioactivity (Table 1). The result is in keeping with the large rate of incorporation encountered with muramine (**3b**). A similarly negative result was obtained on feeding the possible precursors **7** and **8**, two 13-oxygenated muramine derivatives. The synthesis of **7** and **8** is delineated in Scheme 5. Obviously, the presence of any oxygen functions at C-13 inhibits an incorporation of the substance into the rhoeadine **5**. This fact is of interest in connection with a recent biogenetic proposal [24] by which 13-oxygenated compounds like a *N*-quaternary 13-oxo-tetrahydroprotoberberine were suggested to be derived from a quaternary compound such as **2**.

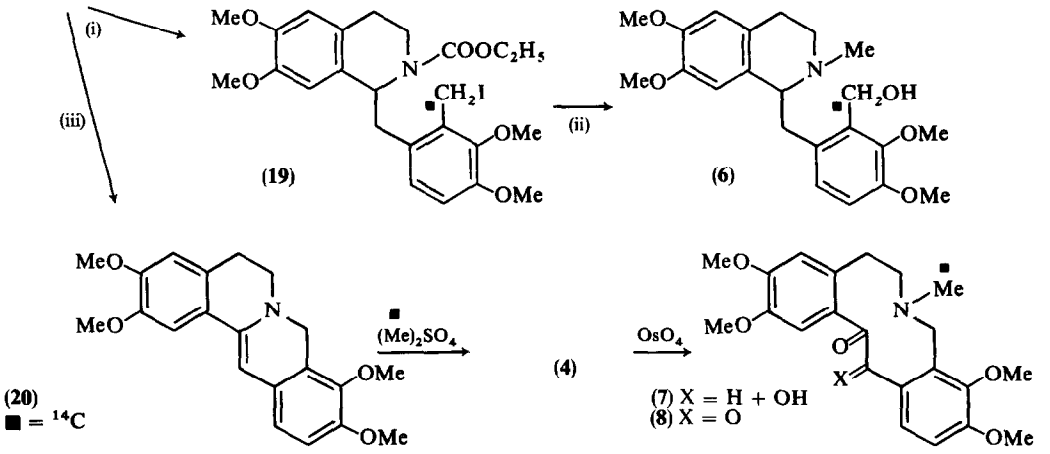
It can be concluded that precursors which deviate from the natural intermediates by even slight structural changes are not utilized in alkaloid biosynthesis. These

Table 4. Degradation of alpinigenine-[^{3}H] from expt 2

	dpm/mM ($\times 10^5$)	% of removed activity found	calculated*
Alpinigenine (**5**) (diluted)	64.4	0	0
Nitrile derivative **12**	51.1	20.7 (from C-14)	21.7
Phthalimidine derivative **13**	0.6	78.3 (from C-2)	78.3

* cf. Scheme 2. The activity of a hydrogen at THP-C-13 relative to that one at THP-C-8 was 34.7:9.6, i.e. 78.3:21.7.

Compound 1



(i) $ClCOOC_2H_5$–NaI–Me_2CO. (ii) NaOAc–Me_2SO; $LiAlH_4$. (iii) $Hg(OAc)_2$; $LiAlH_4$.

Scheme 5. Syntheses of test precursors (4) (6) (7) (8).

facts point to a high degree of specificity in alkaloid metabolism in *P. bracteatum*.

Detection of intermediates by tracer dilution technique

Finally, a kind of trapping experiment was undertaken in which, after a relatively short incubation period using methionine-[^{14}Me], the crude alkaloid fraction obtained was mixed with radioinactive carriers, both **1** and **3b**, to give the radioactive material on separation and careful purification (Table 5). Similarly, the *N*-quaternary intermediate **2** was added to the aqueous mother liquors remaining after the normal work-up procedure and then reisolated. Due to the rather small quantities of the compounds **1**, **2**, and **3b** originally present in the plant, the specific radioactivities encountered in these intermediates are markedly lower if compared with the endproduct **5**, but they are large enough to show clearly that the natural precursors labelled by methionine could be detected in the plant for the first time.

In conclusion, experiments 1 to 7 show that according to Scheme 1 there is no direct conversion of THP into **5** which does *not* include its *N*-methylated derivative **2**. However, while the pathway starting with the amino acid tyrosine can be investigated with respect to almost all of the steps leading to the protopine **3b**, there remains a relatively large gap in our knowledge regarding the intermediates between **3b** and **5**.

EXPERIMENTAL

General. Corrected mp's were determined on a microscope hot stage according to Boetius. If not specified otherwise Merck products were used for TLC and column chromatography, such as Kieselgel G and Si gel 0.05–0.2 mm or Al_2O_3 according to Brockmann (activity III), respectively.

Plant material, technique of feeding experiments, and isolation of alpinigenine (**5**) had been described in the previous communication [6] except for the following modification. To get a better yield of pure **5** rather dark material derived from the column fractions was dissolved in 0.1 M HCl, the acidic soln purified by benzene extraction, and the alkaloid reisolated using Et_2O from the soln adjusted to pH 9.

Counting of radioactivity in general was performed simultaneously by two liquid scintillation counters TriCarb Model 3003 and Model 3380 (Packard). External standardization was used. Each sample was dissolved in 12 ml toluene containing 4 g POP and 0.3 g POPOP per l. So as to provide optimal counting rates and secure a high reproducibility, no more than 1 ml MeOH was usually added to the mixture. The efficiency thus reached was *ca* 30 and 75% with ^{3}H and ^{14}C, respectively. Samples containing both ^{3}H and ^{14}C were examined using the channel discrimination method by which ^{14}C was detected separately with a rate of more than 50% whereas ^{3}H counts had to be corrected for ^{14}C by a calibration curve.

TLC radioscan. 5 mg crude alkaloid fraction was spotted on a 3 cm starting line of a TLC plate which after development with C_6H_6–MeOH (8:2) was analysed on a scanner.

Introduction of tritium into THP (**1**), (a) *THP-[8,14-^{3}H]*.

Table 5. Tracer dilution analyses on the precursors **1**, **2**, and **3b***

	1	**2**†	**3b**	**5**
Inactive precursor (added mg)	30	50	45	—
Radioactive precursor (reisolated mg)	22	45	42	31
Specific activity found (dpm/mM)	7.3×10^5	7.6×10^4	2.9×10^6	4.7×10^7
Rate of incorporation (ppm)	30.3	16.0	214	2520

* The source of radioactivity was methionine-[*S*-^{14}Me], 0.67 mCi, cf. experimental part. † **2**, X = J.

To prepare palmatinium chloride the original Gadamer procedure [25] was modified. A soln of 500 mg **1** in 1.5 ml HOAc was diluted with 10 ml H_2O and filtered. After the addition of 2.5 g $Hg(OAc)_2$ dissolved in 25 ml H_2O plus one drop of HOAc the mixture was kept at 60° for 1 hr. $Hg_2(OAc)_2$ was filtered off from the chilled mixture and excess satd NaCl soln was added to the filtrate. Palmatinium chloride containing an appreciable amount of Hg_2Cl_2 was sucked, washed with water, dried, and found to be apt for a subsequent reduction with $NaBH_4$–[^{3}H]. Crude product (740 mg) was boiled with 600 ml EtOH. The tritiated hydride (8.4 g containing 8.0 g $BaSO_4$) was introduced by 5 portions into the soln decanted from Hg_2Cl_2 and the mixture heated to 60° until the yellow colour faded appreciably (50–100 min). Evaporation to dryness was performed in N_2. Extraction of the residue (Et_2O) afforded **1** which was purified passing an ethereal soln through Al_2O_3 (15 g). Subsequent crystn of the product from EtOH gave 205 mg **1**, mp 147–149° showing constant sp. act. after the third crystn, 1.77×10^9 dpm/mM. A degradation according to Scheme 2 showed that the main fraction of the total activity had been incorporated at C-8 (84.3%) and all the remainder was at C-14 (15.7%).

(b) *THP-[8,13,14-^{3}H;8-^{14}C]*. Crude palmatinium chloride prepared from THP-[8-^{14}C] [6] (200 mg, 4.44×10^8 dpm/mM) as described above was dissolved in 11 ml DMSO distd over calcium hydride and 0.4 ml tritiated H_2O (40 mCi) was added. A reduction using 2 g $NaBH_4$-[^{3}H]-$BaSO_4$ was performed as indicated above except for the time (30 min). After the addition of sufficient MeOH the mixture was filtered to remove $BaSO_4$. Filtrate was concd to a small vol. in N_2, then diluted with 50 ml H_2O and extracted exhaustively with Et_2O. The organic layer was washed 2 × with H_2O, dried (Na_2SO_4) and evapd to dryness. Purification as described above yielded the multiply labelled THP (74 mg), ^{3}H:^{14}C = 6.2; 2.73×10^9:4.44×10^8 dpm/mM.

THP-Methiodide-[8,13,14-^{3}H;8-^{14}C] (**2**). After heating with CH_3I (20 min) the foregoing THP (45 mg) mixed with similarly prepared THP-[8,13,14-^{3}H] (40 mg, 2.60×10^9 dpm/mM) gave a mixture of the α- and β-methiodides **2** (110 mg) which was fed in expt 1 without recrystn prior to use. A specimen was crystd × 3 from MeOH and assayed, ^{3}H:^{14}C = 9.3; 2.44×10^9: 2.62×10^8 dpm/mM; mp 259–261° (lit [11] mp 266°).

Chemical degradation of tritiated THP was performed according to Scheme 2 in order to determine quantitatively the label localized at different positions. As an example the degradation of a sample containing additional ^{14}C as an internal standard will be outlined in detail.

(a) *N-Cyano-14-dihydro-normuramine-[8,13,14-^{3}H;8-^{14}C]* (**10**). The multiply labelled THP (see above) (*ca* 5 mg, ^{3}H:^{14}C = 6.2; 2.73×10^9:4.44×10^8 dpm/mM) was diluted with inactive THP (250 mg) and recrystallized 2×. The latter (186 mg, ^{3}H:^{14}C = 6.3; 6.88×10^7:1.10×10^7 dpm/mM was dissolved in 15 ml THF–H_2O (5:2) and reacted with 109 mg cyanogen bromide in the presence of 44 mg MgO at 45° as described earlier [15] to give 194 mg **10** which after three recrystns showed ^{3}H:^{14}C = 6.3; 6.77×10^7:1.07×10^7 dpm/mM.

(b) *N-Cyan-normuramine-[8,13-^{3}H;8-^{14}C]* (**11**). The cyanamide **10** (170 mg) in pyridine (1.5 ml) was added to a suspension of CrO_3 (88 mg) in 2 ml of the same solvent and the mixture kept at room temp. for 20 hr. After diln with $CHCl_3$ (50 ml) the resulting slurry was stirred with $NaHSO_3$ soln (20%, 2 × 20 ml, 10 min) and satd $NaHCO_3$ soln, then dried and evapd to dryness. Residue in 2 ml CH_2Cl_2 was passed through a column with Al_2O_3 (10 g). The ketone **11** (149 mg) eluted with 60 ml CH_2Cl_2 was crystd several times from MeOH, mp 185–187° (195–196° after drying at 100°): IR ($CHCl_3$, cm^{-1}): 1696 (C=O), 2219 (C≡N); UV, λ_{max}^{EtOH} nm (log ε): 211 (4.50), 225 (4.49), 275 (4.19), and 286 (4.19); MS (probe) 6–16 eV *m/e* (rel. int.): 396 [M$^+$] (36), 353 M − HOCN (61), 164 (100), 149 (70); NMR (60 MHz, $CDCl_3$, TMS): δ 6.90 (1H, *d*, *J* = 9, C-11 or C-12), 6.86 (1H, *s*, C-1), 6.75 (1H, *d*, *J* = 9, C-12 or C-11), 6.55 (1H, *s*, C-4), 4.22 (2H, *s*, C-13), 4.03 (2H, *s*, C-8), 3.83 + 3.82 + 3.78 + 3.77 (12H, 4*s*, 4 × *O*Me), 3.3–2.9 (*ca* 4H, *m*, C-6 + C-5). (Found: C, 66.9; H, 6.3; N, 7.1. $C_{22}H_{24}N_2O_5$ requires: C, 66.6; H, 6.1; N, 7.1%). Radioassay showed ^{3}H:^{14}C = 5.5; 6.01×10^7: 1.10×10^7 dpm/mM.

(c) *N-Cyan-13-oxo-normuramine-[8-^{3}H;8-^{14}C]* (**12**). A dioxane soln (7.5 ml) containing 100 mg **11**, 50.2 mg H_2SeO_3, and 0.5 ml H_2O was refluxed for 16 hr and then kept at room temp. overnight. Residue obtained by evaporation in vac. was treated with 30 ml 0.2 M KOH and extracted (3 × 20 ml CH_2Cl_2). The extract was stirred with 1 M KOH (12 ml, 15 min) washed with H_2O, dried and evaporated to dryness. To remove labile tritium and traces of starting material the resinous mass was chromatographed over 12 g Al_2O_3. Elution with CH_2Cl_2 afforded 82 mg **12**, forming yellow crystals from CH_2Cl_2–Et_2O, mp 217–219°: IR (Nujol, cm^{-1}): 1671 (C=O), 2214 (C≡N); UV, λ_{max}^{EtOH} nm (log ε): 211 (4.52), 235 (*sh* 4.22), 322 (4.06); MS (probe) 6–16 eV *m/e* (rel. int.): 410 [M$^+$] (31), 207 (62), 206 (62), 178 (100), 150 (100); NMR (60 MHz, $CDCl_3$, TMS): δ 7.90 (1H, *d*, *J* = 9, C-12), 7.80 (1H, *s*, C-1), 7.00 (1H, *d*, *J* = 9, C-11), 6.71 (1H, *s*, C-4), 4.45 (2H, *s*, C-8), 3.98 + 3.93 + 3.86 (12H, 4*s*, 4 × *O*Me), 3.4–3.1 (*ca* 4H, *m*, C-6 + C-5). (Found: C, 64.8; H, 5.5; N, 7.1. $C_{22}H_{22}N_2O_6$ requires: C, 64.4; H, 5.4; N, 6.8%). Assay for radioactivity provided ^{3}H:^{14}C = 1.2; 1.30×10^7: 1.10×10^7 dpm/mM.

Syntheses of muramine-[8-^{14}C] (**3b**) *and the test precursors* **6**, **7**, and **8** were performed according to lit. [13] general tracer techniques and precautions being followed. Prior to feeding the labelled products were purified carefully and radioactive uniformity was secured using TLC and subsequent radioactive scanning of the plate.

7,8-Dihydropalmatine (**20**). The original procedure [26] for dihydroberberine was adopted as follows. 2.03 g crude palmatinium chloride prepared from 1 g THP as described above was added with stirring to a slurry of 825 mg $LiAlH_4$ in 120 ml dry Et_2O kept at $5° < t < 10°$. Stirring and chilling were continued for 45 min. Excess of reductant was destroyed cautiously using ice–H_2O and $Al(OH)_3$ was dissolved with 2 M HCl (40 ml). The soln to which was added NaK tartrate (15 g) was adjusted to pH 8 by means of 20% KOH and $NaHCO_3$ and then extracted with C_6H_6 (3 × 120 ml). A TLC assay (C_6H_6–MeOH 8:2) of the dried organic layer (Na_2SO_4) revealed *ca* 60% **20** +25% 1 + 15% palmatine and other polar products. In an identical run with palmatine chloride freed from Hg salts by a tedious procedure [25] overreduction was again observed. Resisting chromatographic separation the product obtained from the benzene extract was purified by a simple recrystn from the same solvent (10 ml). The crystals (500 mg) containing traces of THP were subjected to several crystns from Me_2CO, mp 155–160°: UV, λ_{max}^{EtOH} nm (log ε): 223 (4.43), 276 (4.25), 357 (4.24); MS (probe) 6–16 eV *m/e* (rel. int.): 353 [M$^+$] (100), 338 (62), 142 (62); NMR (60 MHz, $CDCl_3$, TMS): δ 7.13 (1H, *s*, C-1), 6.70 (2H, *s*, C-11 + C-12), 6.56 (1H, *s*, C-4), 5.97 (1H, *s*, C-13), 4.27 (2H, *s*, C-8), 3.86 + 3.81 + 3.78 + 3.76 (12H, 4*s*, 4 × *O*Me), 2.96 (4H, *m*, C-5 + C-6). (Found: C, 71.2; H, 6.6; N, 4.2. $C_{21}H_{23}NO_4$ requires: C, 71.4; H, 6.6; N, 4.0%).

7,8-Dihydropalmatine-methosulphate-[N-^{14}Me]$^+$ [SO_4-^{14}Me]$^-$ (**4**). In an exchange catalysed by NaOMe Me_2SO_4 was equilibrated with ^{14}MeOH to give the labelled dimethyl sulphate [27]. 8 mg CH_3ONa dried in vac. was placed into a small ampoule protected from moisture and CO_2. Chilled ^{14}MeOH (11 mg, 5 mCi) mixed with 500 µl $(Me)_2SO_4$ was cautiously transferred upon the above-mentioned catalyst kept at −70°. The sealed ampoule was heated at 60° for 10 hr to give a brown liquid used immediately for *N*-methylation. The latter (240 µl) was added to a warm soln of **20** (270 mg) in dry C_6H_6 (10 ml) and the mixture was concd slightly (allowing a trace of ^{14}MeOH to be removed) and refluxed for 2 hr. The formed sediment was collected by filtration, washed several times with C_6H_6 and recrystd from MeOH to give 250 mg **4** (X = [SO_4Me]$^-$) (84% th.), mp 231–233°: UV, λ_{max}^{EtOH} nm (log ε): 218 (4.35), 251 (4.13), 353 (4.52), 368 (*sh* 4.42); MS (probe) *m/e* (rel. int.): 368 (M$^+$) (71), 353 (100). Presumably due to excess Me_2SO_4 firmly bound to **4** inadequate C, H, N-values were encountered. Radioassay: 1.76×10^9 dpm/mM. On admixture of inactive material the

product was applied to feeding expt 4 and the synthesis of 13-oxygenated derivatives of muramine [13], respectively.

Dilution analysis on tetrahydropalmatine (**1**), *tetrahydropalmatine methiodide* (**2**), *and muramine* (**3b**). The results comprised in Table 5 were obtained from such a feeding of methionine-[^{14}Me] (33.9 mg, 0.67 mCi) in which according to relatively short time of incubation labelling of intermediary compounds occurring in traces only should be more efficient than in a normal expt. An aq. soln (14 ml) of the precursor was administered to 14 shoots of 3 flowering plants by the usual way. After 30 hr the aboveground parts were harvested (128 g) and the alkaloid fraction was isolated (104 mg). The latter after admixture of 30 mg radioinactive **1** and 45 mg radioinactive **3b** was preseparated on a column of 18 g silica pretreated with C_6H_6–MeOH (9:1), which was run as follows. After passing 20 ml of that solvent further 20 ml provided THP + **5** as a mixture (80 mg). Intermediate fractions containing impure thebaine were obtained using in turn C_6H_6–MeOH (8:2, 45 ml), C_6H_6–$CHCl_3$–MeOH (6:2:2, 15 ml) and (4:4:2, 12 ml). At last $CHCl_3$–MeOH (8:2, 100 ml) recovered dark coloured **3b** (42 mg). Crude **3b** was dissolved in 10 ml 0.1 M HCl, the soln filtered, washed with C_6H_6–Et_2O (1:1, 3 × 10 ml) and made alkaline using NH_3. The base was taken up by Et_2O which was dried (Na_2SO_4) and concd up to 2 ml to leave 11.3 mg **3b** as colourless crystals, mp 176–178°. The sp. act. was unchanged on a second crystn from Me_2CO, 2.9 × 10^6 dpm/mM. The above-mentioned mixture containing THP + **5** in C_6H_6 soln was placed on a column of Al_2O_3 (12 g, activity II), and 40 ml C_6H_6 were passed through. The same solvent (10 ml) and in succession C_6H_6–Et_2O (4:1, 20 ml) recovered 40 mg dark coloured material containing most of the THP previously added to the crude alkaloid fraction. Pure THP (6.8 mg), mp 148–149° (lit. [29] mp 148°); 7.3 × 10^5 dpm/mM, was obtained by a similar acid passage as used above and subsequent crystn from MeOH. Finally, C_6H_6–Et_2O (1:1, 40 ml) provided alpinigenine (**5**) (37 mg) which too was purified in the same manner as outlined above. Crystn from C_6H_6–MeOH afforded colourless crystals (14.6 mg), mp 186–187°; 4.7 × 10^7 dpm/mM (lit. [3] mp 186.5–187°). Radioactive *N*-quaternary precursor **2** (X = *J*, 55 mg) was added to the aq. layer left from the isolation of the crude alkaloid fraction. The soln acidified with 50% HJ (5 ml) was concd up to 30 ml in vac. Crystals obtained on standing were recrystd 4 × from MeOH, mp 260–262° and identified by IR as THP-β-methiodide. Unfortunately, fading from initial *ca* 550 dpm/mg to 120 dpm/mg after the last crystn the sp. act. could not be brought to constancy in this way. A method considering the radioactivity of both diastereomeric forms of **2**, α and β, was found in applying Hofmann degradation as follows. The crystals were unified with material collected from mother liquors. An aq. soln (15 ml) was stirred with excess Ag_2O for 2 hr to give the methohydroxide **2** (X=OH) after filtering and evaporation under red. pres. The remainder in 8 ml 40% KOH was heated under reflux for two hr and subsequently worked up in the usual way using Et_2O. The product (32 mg) according to TLC (C_6H_6–MeOH, 4:1) consisted of *ca* 98% methine B (styrol derivative) accompanied by traces of the stilbene methine A [11]. It was purified and characterized as hydrochloride, which was proven radiochemically pure after two crystns from MeOH–Et_2O and a further one from Me_2CO, mp 205–209° (dec.); 76 000 dpm/mM; identical in every respect (mp, mixed mp, IR) with authentic material prepared from inactive **2** (mp 206–210°, dec.; lit. [11] mp 210°, dec.).

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