Specimens of plasma from tolazamide-treated guinea pigs (single oral dose of 15 mg. drug/kg. body weight) were extracted and analyzed in the same manner as described for the plasma standards. The concentration of tolazamide was determined from the standard curve. A peak plasma tolazamide concentration of 12.5 mcg./ml. was observed at 1 hr. after oral drug administration, indicating rapid drug absorption. A plot of the plasma concentrations of tolazamide (expressed in micrograms per milliliter) *versus* time (in hours) is presented in Fig. 4. By using the data between 1 and 14 hr., the plasma drug disappearance half-life was graphically estimated to be 2.7 hr. (Fig. 5).

During the course of this work, Sabih and Sabih (4) published a GC method for the determination of tolbutamide (VIII) and chlorpropamide (IX). These authors claimed to have analyzed the intact methyl derivatives of these drugs by GC. However, they did not establish the identity of the chromatographic species either by collecting a sample of the effluent or by GC–mass spectrometry. Considering the high operating temperatures reported (4) (flash heater at 330° and column temperature at 210°), the thermal fragmentation of the methyl derivatives cannot be excluded.

The present GC method described for the determination of tolazamide (I) in guinea pig plasma is better than the previously reported methods (1–3, 5) since: (*a*) it has greater detection sensitivity [the lower limit being 0.7 mcg. tolazamide/ml. plasma rather than 20 mcg./ml. plasma (2) or ~3 mcg./ml. plasma (3)], and (*b*) it has greater specificity on account of the preliminary TLC purification step which is capable of resolving intact tolazamide from its metabolites as well as from plasma contaminants. The method could be conveniently adapted for determination of other related sulfonylureas in pharmaceutical preparations as well as a variety of biological specimens.

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Opium Alkaloids X: Biosynthesis of 1-Benzylisoquinolines

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Abstract ☐ Radioactively labeled norreticuline was incorporated into papaverine in *Papaver somniferum* to an extent of about 5% without prior demethylation to norlaudanosoline. 1,2-Dehydronorreticuline does not participate in the biosynthesis of this alkaloid. (+)-Laudanosine is derived from (+)-reticuline *via* (+)-laudanidine, while (+)-codamine plays only a minor role. Radiodilution studies indicated that tetrahydropapaverine and papaveroline 6,3′,4′-trimethyl ether (pacodine) occur in the opium poppy. Based on the results of a series of tracer experiments, a scheme was proposed for the biosynthesis of benzylisoquinoline alkaloids in *P. somniferum*.

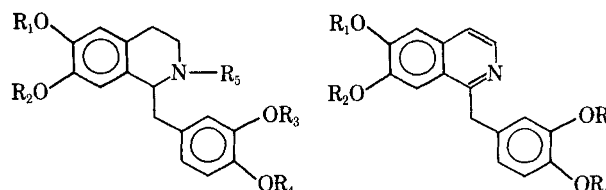
Keyphrases ☐ 1-Benzylisoquinolines—proposed biosynthesis in *P. somniferum* ☐ Biosynthesis—1-benzylisoquinolines in *P. somniferum*, proposed ☐ *Papaver somniferum*—proposed biosynthesis of 1-benzylisoquinolines ☐ Radioactive labeling—norreticuline, biosynthesis in *P. somniferum* ☐ Opium alkaloids—proposed biosynthesis of 1-benzylisoquinolines

The benzylisoquinoline alkaloids are widely distributed in the *Papaveraceae* family, occurring most extensively as the 1,2,3,4-tetrahydro derivatives. Four alkaloids of this general structure have been isolated from the opium poppy or from its dried latex, namely, codamine (I), laudanidine (II), laudanosine (III), and reticuline (IV). Two of these are present in both enantiomeric forms (II and IV) with an excess of one of the optical isomers [(−)-laudanidine and (+)-reticuline]. Norreticuline (V) has not yet been isolated but was shown to be an effective precursor of morphine and codeine (1).

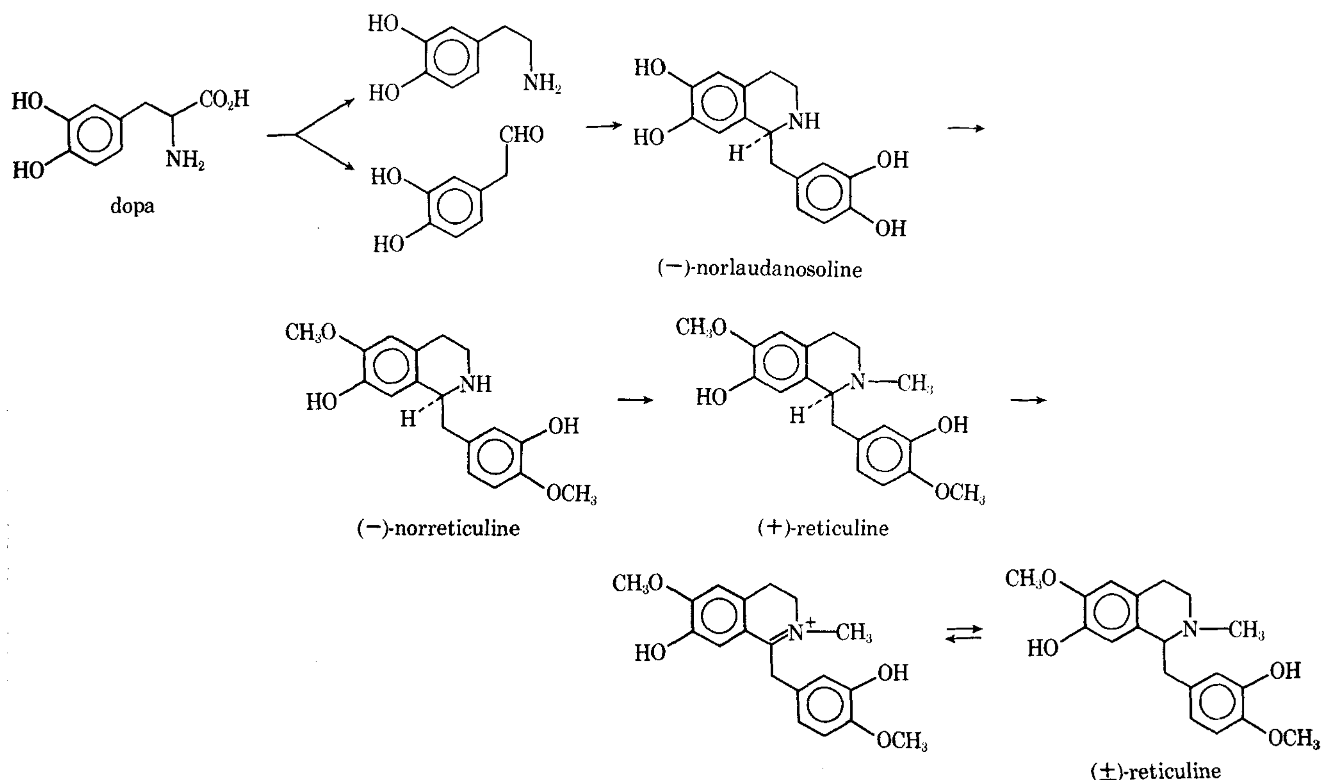
The oxidized, fully aromatic benzylisoquinolines are not as common; two such alkaloids, papaverine (VIII) and palaudine (IX), were isolated from opium (2).

PREVIOUS WORK

In 1910, Winterstein and Trier (3) proposed that the benzylisoquinoline system is derived biosynthetically from two units of 3,4-dihydroxyphenylalanine (dopa) which, by decarboxylation and oxidative deamination, were believed to give rise to 3,4-dihydroxyphenethylamine (dopamine) and 3,4-dihydroxyphenylacetaldehyde, respectively. Formation of a Schiff's base and ring closure would give the substance known as norlaudanosoline (tetrahydropapaveroline) (VI). Modern theories (4) consider the reacting units to be



I: $R_1 = R_3 = R_4 = R_5 = CH_3$, $R_2 = H$
II: $R_1 = R_2 = R_4 = R_5 = CH_3$, $R_3 = H$
III: $R_1 = R_2 = R_3 = R_4 = R_5 = CH_3$
IV: $R_1 = R_4 = R_5 = CH_3$, $R_2 = R_3 = H$
V: $R_1 = R_4 = CH_3$, $R_2 = R_3 = R_5 = H$
VI: $R_1 = R_2 = R_3 = R_4 = R_5 = H$
VII: $R_1 = R_2 = R_3 = R_4 = CH_3$, $R_5 = H$
VIII: $R_1 = R_2 = R_3 = R_4 = CH_3$
IX: $R_1 = R_2 = R_4 = CH_3$, $R_3 = H$



Scheme I—Proposed biosynthesis of (±)-reticuline

dopamine and 3,4-dihydroxyphenylpyruvic acid. There is considerable evidence that norlaudanosoline is the primary substance from which the other benzylisoquinolines are derived by dehydrogenation and/or methylation. Biosynthetic methylations do not usually occur at random but in a definite sequence. Furthermore, they show a great deal of stereoselectivity. Tracer experiments suggested that natural norlaudanosoline in the opium poppy is the levorotatory isomer (5). It undergoes *O*-methylation to (−)-norreticuline, which is further methylated to (+)-reticuline. (+)-Reticuline is capable of undergoing racemization *via* an oxidation–reduction system, which presumably involves 1,2-dehydroreticuline (5) (Scheme I). The levorotatory isomer thus produced is utilized for biosynthesis of the hydrophenanthrene alkaloids. (+)-Reticuline, on the other hand, is required for the biosynthesis of aporphines, protoberberines, protopines, and phthalide-isoquinolines in the opium poppy. (+)-Norlaudanosoline, which on methylation would produce (−)-reticuline directly, cannot serve as a precursor of this important alkaloid (5).

Papaverine—Earlier experiments showed that tyrosine (a precursor of dopa) and norlaudanosoline are effectively incorporated into papaverine (1, 6, 7). However, nothing is known with regard to the sequence of the methylation steps and the dehydrogenation necessary to transform norlaudanosoline to papaverine. There is also a question as to the mechanism of dehydrogenation. It might proceed stepwise or by a concerted process. Battersby *et al.* (8) showed that 1,2-dehydronorreticuline can serve as a precursor of morphine.

Incorporation of this compound into papaverine would give some measure of support to the possibility of a stepwise dehydrogenation. To study these questions, feeding experiments were performed with radioactively labeled norreticuline and 1,2-dehydronorreticuline. Papaverine was isolated and purified to constant radioactivity, and the position of the label was ascertained by controlled degradation.

Laudanosine—In general, biosynthetic reactions are stereospecific and, as a rule, only one of the optical isomers is formed. Reticuline is the only alkaloid in the opium poppy that has been shown to undergo racemization in the plant. This process serves a very important biosynthetic function as a prerequisite for the production of morphine alkaloids. Since laudanidine exists in the plant in both enantiomeric forms, one may surmise that these are derived directly from the respective isomers of reticuline by methylation of the phenolic hydroxyl group in the 7-position. The steric relationship of this hydroxyl group to the basic nitrogen is not affected by the configuration of the asymmetric center at C-1, and both isomers may be expected to fit the active site of the enzyme. Methylation of the hydroxyl function in the 3′-position is more stereoselective. It appears that only the L-structures have the proper configuration to fit the enzyme surface to give (+)-codamine and (+)-laudanosine. It was of interest to determine whether (+)-codamine, (+)-laudanidine, or both can serve as precursors of laudanosine. To this end, radioactively labeled (±)-codamine and (±)-laudanidine (laudanine) were administered to separate batches of *Papaver somniferum*. (±)-Laudanosine was added as a "cold" carrier during the extraction, isolated, purified to constant radioactivity, and subjected to controlled degradation.

RESULTS AND DISCUSSION

The names and amounts of labeled precursors and the results of the feeding experiments are given in Table I. Administration of (±)-norreticuline-(3-^{14}C) (XI) gave good incorporation into papaverine (XIX). Controlled degradation was performed by *N*-methylation, reduction to laudanosine with sodium borohydride, Hoffmann degradation, and ozonolysis, as described by Battersby and Harper (6). The carbon atom in the 3-position was recovered as formaldehyde, which was purified as the dimedone derivative. Since practically all radioactivity (93%) resided in this position, incorporation of norreticuline into papaverine had not involved any randomization of the label. This experiment did not prove that norreticuline was an obligatory precursor of papaverine.

It was conceivable for norreticuline to undergo demethylation to norlaudanosoline in the plant before being dehydrogenated. The experiment was, therefore, repeated with (±)-norreticuline-(6-*O*-^{14}CH$_3$). The Indra variety of *P. somniferum* used in 1969 proved to be free of papaverine, and no radioactivity could be detected in the alkaloid isolated after addition of the "cold" carrier. The Noordster variety is rich in papaverine, and addition of carrier is not necessary. When norreticuline-(6-*O*-^{14}CH$_3$) was administered to these plants, good incorporation of radioactivity into papaverine was obtained. A Zeisel *O*-demethylation recovered all radioactivity as triethylmethylammonium iodide. 1,2-Dehydronorreticuline-(3-^{14}C) was not incorporated into papaverine and is, therefore, not an intermediate in a stepwise dehydrogenation to the aromatic

Table I—Results of Feeding Labeled Precursors to *Papaver somniferum*

Precursor	Amount Administered, μc.	Number of Plants	Year	Variety	Alkaloid Added as Carrier	Alkaloid Isolated	Incorporation, %	Degradation, Percent of Radioactivity Retained
(±)-Reticuline-(3-^{14}C)	45	9	1966	Noordster	None	Papaverine	0.07	—
1,2-Dehydronorreticuline-(3-^{14}C)	11.5	5	1967	Noordster	None	Papaverine	0.00034	—
(±)-Norreticuline-(3-^{14}C)	14.5	3	1967	Noordster	None	Papaverine	1.47	93
(±)-Norreticuline-(6-*O*-^{14}CH$_3$)	49.2	10	1969	Indra	Papaverine	Papaverine	0.0008	—
(±)-Norreticuline-(6-*O*-^{14}CH$_3$)	19.7	7	1970	Noordster	None	Papaverine	4.95	102
(±)-Codamine-(3-^{14}C)	11	4	1967	Noordster	(±)-Laudanosine	(±)-Laudanosine	0.036	—
(±)-Laudanidine-(*N*-^{14}CH$_3$)	44.3	7	1969	Indra	(±)-Laudanosine	(±)-Laudanosine	0.155	99
(±)-Laudanidine-(*N*-^{14}CH$_3$)	43.4	7	1970	Noordster	(±)-Laudanosine	(±)-Laudanosine	2.032	104
(±)-Norlaudanosoline-(3-^{14}C)	11.2	10	1970	Noordster	(±)-Tetrahydropapaverine	(±)-Tetrahydropapaverine	0.868	—
(±)-Norreticuline-(6-*O*-^{14}CH$_3$)	19.7	7	1970	Noordster	Pacodine	Pacodine	0.017	—

benzylisoquinolines, nor is it reduced to norreticuline in the plant. The incorporation of 1,2-dehydronorreticuline into morphine observed by Battersby *et al.* (8) must involve *N*-methylation and reduction to reticuline, in that order.

The slight, but still significant, incorporation of reticuline into papaverine is indicative of *N*-demethylation. Biosynthetic *N*-demethylation of alkaloids does not appear to occur to any great extent in the way that *O*-demethylations do. However, it was reported by Barton *et al.* (9) for a quaternary alkaloid (tembetarine = *N*-methylreticuline). Whether the *N*-demethylation observed in this feeding experiment with reticuline takes place before or after aromatization is still subject to question. By the reverse isotope dilution technique, it was found that (±)-norlaudanosoline-(3-^{14}C) (X) gave good incorporation of radioactivity into tetrahydropapaverine (XIV), and (±)-norreticuline-(6-*O*-^{14}CH$_3$) (XI) conferred a small but measurable amount of radioactivity to papaveroline 6,3′,4′-trimethyl ether (pacodine) (XVIII). The amounts of these alkaloids remaining after purification to constant radioactivity were too small to permit determination of the position of the label, and the experiments will be repeated. Although tetrahydropapaverine might be an intermediate between norreticuline and papaverine, it is more likely that aromatization occurs at the norreticuline stage. The presence of palaudine (XVII) and pacodine (XVIII) lends support to this view. Both alkaloids are present in low concentrations, indicative of a fast turnover, presumably by methylation to papaverine. Work is in progress to detect the hypothetical papaveroline 6,4′-dimethyl ether and to determine its effectiveness as a precursor of papaverine.

(±)-Laudanidine-(*N*-^{14}CH$_3$) (XIII) was incorporated well into laudanosine (XVI). Selective demethylation showed that all radioactivity resided in the *N*-methyl group. (±)-Codamine (XV) gave only a low degree of incorporation. Therefore, (+)-laudanidine may be looked upon as the immediate precursor of laudanosine, while the codamine route represents only a minor pathway. This also tends to explain the presence of an excess of (−)-laudanidine, which appears to be an end-product in the biosynthesis.

Based on the experimental results discussed here, the proposed biosynthetic pathways of the benzylisoquinoline alkaloids are summarized in Scheme II.

EXPERIMENTAL

The methods used for the determination of radioactivity, cultivation of plants, and administration of labeled precursors were described in a previous publication (10).

Synthesis of Labeled Precursors—Several practice runs were always made with nonradioactive materials prior to the synthesis of the labeled compounds. The intermediates were characterized by TLC, GLC, NMR, IR spectroscopy, and melting points. The final products were compared with authentic natural alkaloids when available.

1,2-Dehydronorreticuline-(3-^{14}C) Hydrochloride—1-(3-Benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxy-[3-^{14}C]-isoquinoline hydrochloride (20 mg.), prepared as described earlier (10), was dissolved in 0.5 ml. of ethanol and 0.5 ml.of concentrated hydrochloric acid. The solution was refluxed for 1 hr. and evaporated to dryness. The residue was crystallized from a mixture of ethanol and ether to give 8.2 mg. of 1,2-dehydronorreticuline-(3-^{14}C) hydrochloride, m.p. 208–210°.

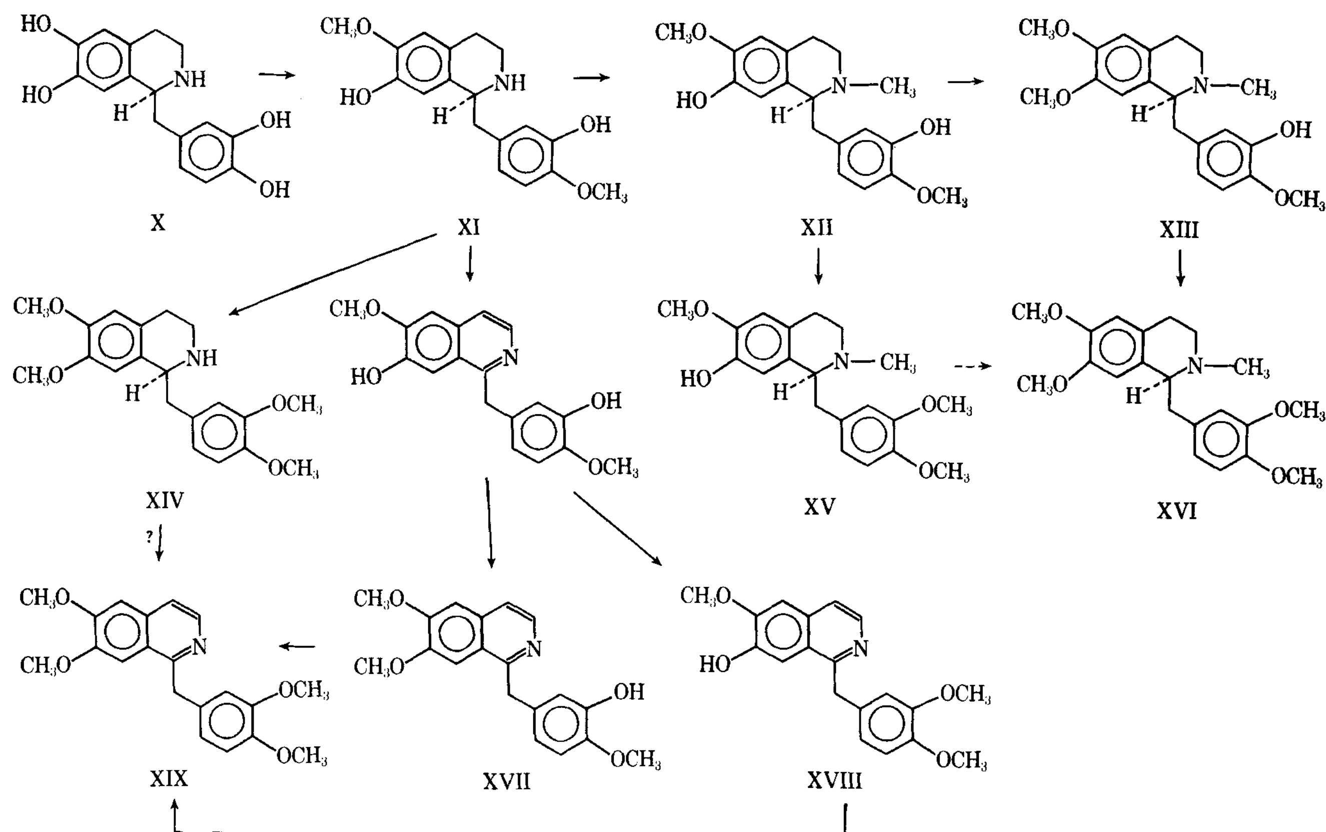
Anal.—Calc. for $C_{18}H_{20}ClNO_4$: C, 61.80; H, 5.72; N, 4.00. Found: C, 61.27; H, 5.61; N, 3.96.

Specific activity = 0.48 mc./mmole.

(±)-Norreticuline-(3-^{14}C) Hydrochloride—1-(3-Benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxy-[3-^{14}C]-isoquinoline hydrochloride (21 mg.) was dissolved in methanol (2 ml.), and 30 mg. of sodium borohydride was added in portions. After about 12 hr. of stirring, the reaction mixture was evaporated to dryness, and 20 ml. of water and 0.5 ml. of 2 *N* sodium hydroxide were added. The solution was extracted with chloroform, and the combined chloroform extracts were evaporated to give a residue of *O*,*O*-dibenzylnorreticuline-(3-^{14}C). It was dissolved in 0.5 ml. of ethanol and 0.5 ml. of concentrated hydrochloric acid, refluxed for 1 hr., and evaporated to dryness. The residue was crystallized from a mixture of ethanol and ether. The substance was identified by TLC and GLC with (±)-norreticuline prepared previously (10) with nonradioactive materials.

(±)-Norreticuline-(6-O-^{14}CH$_3$) Hydrochloride—3-Hydroxy-4-benzyloxybenzaldehyde was prepared by refluxing 3,4-dihydroxybenzaldehyde (4 g.), benzyl chloride (3.5 ml.), and anhydrous potassium carbonate (15 g.) in 150 ml. of acetone for 20 hr. The reaction mixture was chromatographed on a column of silica gel (Woelm, activity II) with benzene. The fractions containing 3-hydroxy-4-benzyloxybenzaldehyde were concentrated under reduced pressure to yield pale-yellow needles, which were recrystallized from benzene; yield 1.64 g.; m.p. 119–123° [lit. (11) m.p. 122°, from ethanol]. The compound was further identified by methylation with methyl iodide and potassium carbonate in acetone to give 3-methoxy-4-benzyloxybenzaldehyde, m.p. 65°. It showed no melting-point depression when mixed with benzylvanillin but showed a 10–12° depression when mixed with benzylisovanillin.

3-Hydroxy-4-methoxybenzaldehyde (620 mg.) was suspended in 10 ml. of anhydrous methanol; a solution of sodium methoxide, prepared from 60 mg. of metallic sodium and 10 ml. of absolute methanol, was added dropwise with stirring. The resulting solution was evaporated to dryness under reduced pressure, and the residue



Scheme II—Proposed biosynthetic pathways of benzylisoquinolines in Papaver somniferum

was suspended in 8 ml. of dry, redistilled dimethylformamide. Radioactive iodomethane (141 mg., 2 mc.) was distilled into the suspension through a vacuum manifold system. The reaction vessel was sealed, and the mixture was allowed to stand at room temperature for 20 hr. It was then heated in a water bath at about 65° for 24 hr. with occasional shaking. The seal was broken, and an excess of iodomethane (4 ml.) was added. The container was sealed again, heated at 65° for 3 hr., and allowed to stand overnight at room temperature. The reaction mixture was transferred quantitatively to a separator with the aid of 5 ml. of benzene and 70 ml. of 0.1 *N* sodium hydroxide and shaken vigorously. The aqueous layer was extracted with 4 × 5 ml. of benzene, and the combined benzene extracts were evaporated to dryness. The oily residue was crystallized from isopropyl ether to give 363 mg. of 3-methoxy-(^{14}C)-4-benzyloxybenzaldehyde, m.p. 65°. The aldehyde was reacted with nitromethane to give 3-methoxy-(^{14}C)-4-benzyloxynitrostyrene and reduced with lithium aluminum hydride to 3-methoxy-(^{14}C)-4-benzyloxyphenethylamine, and the synthesis was continued as described previously (10). The substance was identical by TLC and GLC with synthetic nonradioactive (±)-norreticuline. Specific activity = 1.02 mc./mmole.

(±)-Codamine-(3-^{14}C)—The synthesis of codamine-(3-^{14}C) was similar to that of reticuline-(3-^{14}C) (10), 3,4-dimethoxyphenylacetic acid being used instead of 3-benzyloxy-4-methoxyphenylacetic acid. The synthetic compound was identical with natural codamine isolated from opium (12) by TLC, GLC, and IR spectroscopy. Specific activity = 0.51 mc./mmole.

(±)-Norlaudanosoline-(3-^{14}C) Hydrochloride—The synthesis was carried out as described by Battersby *et al.* (1). The product had a melting point of 278–281° [lit. (1) m.p. 278–281°] and a specific activity of 0.36 mc./mmole.

(±)-Laudanidine-(N-^{14}*CH$_3$*)—1-(3-Benzyloxy-4-methoxybenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline hydrochloride (467 mg.), prepared as described by Deulofeu (13), was suspended in water; nitrogen was bubbled through the suspension, and sodium bicarbonate was added to pH about 8–9. The mixture was extracted with ether (4 × 20 ml.), and the combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in 5 ml. of benzene and transferred to a methylation vessel, 1 cm. in diameter and 10 cm. long. It was connected to a vacuum manifold system through which radioactive iodomethane-(^{14}C) (141 mg., 2 mc.) was distilled into the benzene solution. The reaction vessel was sealed off and left at room temperature overnight. An oily film appeared on the wall of the tube and turned crystalline. After 4 days, an excess of nonradioactive iodomethane (4 ml.) was added, and the mixture was allowed to stand at room temperature for 24 hr. The crystals were collected and suspended in 30 ml. of absolute methanol, and 1 g. of sodium borohydride was added in small portions and with stirring. The yellow suspension turned colorless. After 12 hr. at room temperature, the solution was acidified with 5 ml. of 2 *N* hydrochloric acid and refluxed for 1 hr. Water (50 ml.) was added, and the solution was extracted with ether. The aqueous layer was basified with ammonium hydroxide to pH 9 and extracted repeatedly with ether. The combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness, and the residue crystallized from a mixture of ethanol and chloroform.The crystalline compound was identical with natural laudanine isolated from opium by TLC, GLC, and IR spectroscopy. Specific activity = 1.53 mc./mmole.

(±)-Reticuline-(3-^{14}C)—The synthesis was described in a previous communication (10).

Alkaloids for Carrier Dilution—*(±)-Laudanosine*—This compound was obtained commercially[1].

(±)-Tetrahydropapaverine (Norlaudanosine)—This compound was synthesized as described by Battersby *et al.* (1). The hydroiodide salt melted at 255–256° [lit. (1) 256°].

Papaveroline 6,3',4'-Trimethyl Ether (Pacodine)—The synthesis was carried out as described by Brochmann-Hanssen and Hirai (2).

Extraction and Separation of Alkaloids—The extraction of the plant material and the isolation of the total alkaloids followed the procedure described earlier (10).

Papaverine and Pacodine—The acidic, aqueous solution containing the total alkaloids was extracted with chloroform (4 × 300 ml.), and the combined chloroform extracts were evaporated to dryness.

[1] Mallinckrodt Chemical Works.

The residue was dissolved in a minimum amount of chloroform and chromatographed on a column of silica gel (Woelm, activity I) with a mixture of chloroform and ethanol (98:2). The fractions containing papaverine were combined and evaporated to dryness. The residue was further purified by preparative TLC on silica gel, with benzene–ethanol (4:1) as the developing solvent. The band containing papaverine was scraped off and extracted with warm methanol. Evaporation of the methanol gave a pale-brown residue, which was crystallized from ether to give papaverine, m.p. 146–147°, of constant radioactivity.

After extraction of papaverine, the acidic, aqueous layer was basified with ammonium hydroxide to pH 8–9 and extracted with chloroform. Evaporation of chloroform gave a residue, which was chromatographed on a column of silica gel (Woelm, activity IV) with chloroform. The fractions containing pacodine were combined and evaporated to dryness. The residue was crystallized from methanol to give pacodine of constant radioactivity.

Laudanosine—The acidic, aqueous solution of the total alkaloids was made alkaline with 10% sodium hydroxide to pH 14 and extracted with ether (8 × 100 ml.). The combined ether extracts were washed with water (3 × 10 ml.), dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was chromatographed on a column of neutral aluminum oxide (Woelm, activity IV) with a mixture of chloroform and isopropyl ether (1:1). The fractions containing laudanosine were combined and evaporated to dryness. The residue was crystallized from ether to give laudanosine of constant radioactivity, m.p. 115–116° [lit. (14) m.p. 114–115°].

Tetrahydropapaverine—The acidic, aqueous solution containing the total alkaloids was extracted with chloroform (3 × 200 ml.). The aqueous layer was basified with sodium hydroxide to pH 14 and extracted with a mixture of chloroform and isopropyl alcohol (3:1). The extract was evaporated to dryness; the residue was dissolved in a minimum amount of chloroform, transferred to a column of neutral aluminum oxide (60 g.), and eluted with chloroform–methanol (98:2). The fractions containing tetrahydropapaverine were combined and evaporated to dryness. The residue was subjected to preparative TLC on silica gel with chloroform–methanol (9:1). The band corresponding to tetrahydropapaverine was extracted with methanol. Evaporation of the solvent gave a residue which was dissolved in ether. Addition of hydriodic acid caused precipitation of the hydroiodide, which was recrystallized from water to constant radioactivity, m.p. 255–256° [lit. (1) m.p. 256°].

Degradation of Papaverine—Papaverine (33 mg.), isolated from the plants to which (±)-norreticuline-(3-^{14}C) had been administered, was reacted with iodomethane to give the corresponding methiodide, which was reduced with sodium borohydride in methanol to (±)-laudanosine. This compound was quaternized with iodomethane and subjected to Hoffmann degradation to give a mixture of *cis*- and *trans*-laudanosine methines. Methylation of the mixture with iodomethane gave a yellow residue of *cis*- and *trans*-laudanosine methine methiodide (6). The methiodide was suspended in 5 ml. of water; freshly prepared silver oxide (from 150 mg. of silver nitrate) was added, and the mixture was shaken for 1.5 hr. and filtered. The filtrate was made strongly alkaline with 1 g. of potassium hydroxide, refluxed for 2 hr., cooled, and extracted with chloroform (3 × 20 ml.). The combined chloroform extracts were evaporated to dryness under reduced pressure. The pale-yellow residue (11 mg.) was dissolved in 3 ml. of ethyl acetate, and the solution was cooled in a dry ice–acetone bath. Ozone was passed into it for 20 min., followed by oxygen for 5 min., and the reaction mixture was evaporated to dryness. To the residue were added 8 ml. of water, 90 mg. of zinc dust, and 5 mg. of silver nitrate. The mixture was refluxed for 0.5 hr. and then distilled into a solution of 60 mg. of dimedone in 1.2 ml. of ethanol and 3 ml. of water. When one-half of the volume had distilled over, an equal volume of water was added to the flask and the distillation was continued. Long needles of the formaldehyde–dimedone derivative formed in the receiving flask; they were collected, washed thoroughly with cold distilled water, and crystallized from a mixture of methanol and water to constant radioactivity.

Papaverine, isolated from plants to which (±)-norreticuline-(6-*O*-$^{14}CH_3$) had been administered, was subjected to Zeisel *O*-demethylation as follows. Papaverine (10 mg.), 50 mg. of ammonium iodide, 500 mg. of phenol, 5 drops of chloroauric acid (0.1 g. in 3.5 ml. of water), and 2 ml. of hydriodic acid (freshly distilled from red phosphorus) were placed in the flask of a modified Clark alkoxy apparatus. The mixture was allowed to stand at room temperature for 20 min. in a current of nitrogen and then was heated gradually to 180° in a bath of Wood's metal. The nitrogen gas carrying liberated methyl iodide passed through a trap containing a mixture of 5% sodium thiosulfate and 5% cadmium sulfate (1:1) into a solution of 10% triethylamine in ethanol (5 ml.). The receiving vessel was immersed in a bath of dry ice and acetone. The temperature of the Wood's metal bath was maintained at 180–195° for 1 hr. The triethylamine solution was allowed to stand overnight at room temperature before it was evaporated to dryness. The residue of triethylmethylammonium iodide (28 mg.) was crystallized from ethanol and ether to constant radioactivity.

Degradation of Laudanosine—Laudanosine, isolated from the plants to which (±)-laudanidine-(*N*-$^{14}CH_3$) had been administered, was first *O*-demethylated as described previously. When the reaction was complete—as indicated by formation of the theoretical amount of triethylmethylammonium iodide—the triethylamine solution in the receiving vessel was replaced; the temperature of the Wood's metal bath was raised slowly to 360° where it was maintained for 1 hr. for *N*-demethylation. After 8 hr. at room temperature, the triethylamine solution was evaporated to dryness and the residue was crystallized to constant radioactivity.

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