Biosynthesis of some Alkaloids of *Punica granatum* and *Withania somnifera* [1]

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The biosynthesis of isopeletierine [1-(2-piperidyl)propan-2-one], *N*-methylisopelletierine, and pseudopelletierine (9-methyl-9-azabicylo[3,3,1]nonan-3-one) in intact *Punica granatum* plants has been studied. *N*-Methylisopelletierine is shown to be derived from lysine and acetate. Acetate is shown to be the precursor of the three-carbon unit in isopelletierine and pseudopelletierine. *N*-Methylisopelletierine is shown to be the immediate precursor of psueudopelletierine.

The derivation of anaferine from lysine and acetate has been demonstrated in intact *Withania somnifera* plants. Isopelletierine has also been shown to be a precursor of anaferine.

Punica granatum and *Withania somnifera* contain a number of structurally related piperidine alkaloids: isopelletierine (V), *N*-methylisopelletierine (VI), and pseudopelletierine (IX) in *P. granatum*,[2] and anaferine (VIII) in *W. somnifera*.[3] Isopelletierine and *N*-methylisopelletierine are structurally related to the hemlock alkaloids, coniine and *N*-methylconiine, which have been shown to be of polyketide origin.[4] More recently it has also been shown that pinidine is derived from acetate in *Pinus jeffreyi*,[5] a result in agreement with our findings that pinidine and 2-methylpiperidine are acetate-derived in P. sabiniana.[6] Preliminary results [1,7] point to the pomegranate and withania alkaloids being derived from lysine and acetate, in agreement with the classical hypothesis of Robinson.[8]

The role of lysine in the biosynthesis of the piperidine nucleus of several alkaloids has been established by tracer experiments. [2-^{14}C]Lysine is incorporated into the piperidine nucleus of anabasine.[9] Again, 2,3,4,5-tetrahydro[2-^{14}C]pyridine is incorporated unsymmetrically into this alkaloid.[10] Carbon-2 and carbon-6 of the hetero-ring of sedamine are specifically derived from this precursor.[11] The piperidine nucleus of piperidine-2-carboxylic acid is also derived unsymmetrically from lysine.[12] Recently, the incorporation of radioactivity from [6-^{14}C]lysine into carbon-6 of *N*-methylisopelletierine in *Sedum sarmentosum* has been reported.[13] In each of these alkaloids the ε-amino-group of lysine furnishes the nitrogen of the heterocyclic nucleus.[12,14]

The three-carbon unit of *N*-methylisopelletierine has recently been shown to arise from acetate.[15]

In the pyrrolidine series of alkaloids we have demonstrated the precursor role of hygrine in the biosynthesis of cuscohygrine and hyoscyamine.[16] We consider that the piperidine analogues, anaferine and pseudopelletierine, should be formed from isopelletierine and *N*-methylisopelletierine respectively.

We now outline a biosynthetic scheme (Scheme 1) for these alkaloids which is compatible with the studies mentioned. Lysine (I) undergoes α-oxidative deamination to yield 6-amino-2-oxohexanoic acid (II) which on cyclisation and decarboxylation yields 2,3,4,5-tetrahydropyridine (III). Condensation of this with acetoacetyl-coenzyme A (IV) yields isopelletierine (V); this reaction has been achieved *in vitro*.[17] Methylation of isopelletierine yields *N*-methylisopelletierine (VI). Condensation of isopelletierine (V) with another mole-

[1] Preliminary communication, D. G. O'Donovan and M. F. Keogh, *Tetrahedron Letters*, 1968, 265.
[2] C. Tanret, *Compt. rend.*, 1880, **90**, 695. K. Hess, *Ber.*, 1917, **50**, 368.
[3] A. E. Schwarting, J. M. Bobbit, A. Rother, C. K. Atal, K. L. Khanna, J. M. Leary, and W. G. Walker, *Lloydia*, 1963, **26**, 258.
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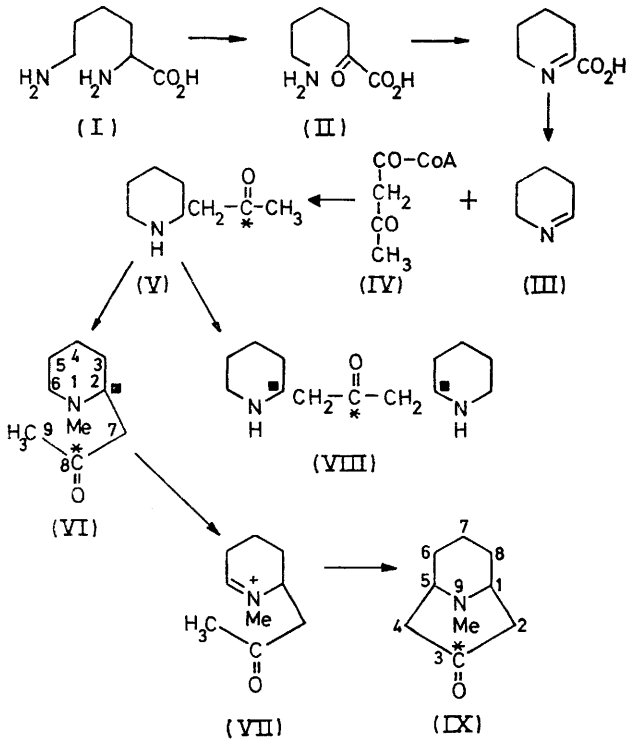
[6] D. G. O'Donovan and N. Morgan, unpublished results.
[7] R. N. Gupta and I. D. Spenser, *Chem. Comm.*, 1968, 85.
[8] R. Robinson, 'Structural Relations of Natural Products,' Clarendon Press, Oxford, 1955, p. 119.
[9] E. Leete, *J. Amer. Chem. Soc.*, 1956, **78**, 3520.
[10] E. Leete, *J. Amer. Chem. Soc.*, 1969, **91**, 1679.
[11] R. N. Gupta and I. D. Spenser, *Canad. J. Chem.*, 1967, **45**, 1275.
[12] R. N. Gupta and I. D. Spenser, *J. Biol. Chem.*, 1969, **244**, 88.
[13] R. N. Gupta and I. D. Spenser, *Phytochemistry*, 1969, **8**, 1937.
[14] E. Leete, E. G. Gros, and T. G. Gilbertson, *J. Amer. Chem. Soc.*, 1964, **86**, 3907.
[15] H. W. Liebisch, N. Marekov, and H. R. Schutte, *Z. Naturforsch*, 1968, **23b**, 1116.
[16] D. G. O'Donovan and M. F. Keogh, *J. Chem. Soc.* (*C*), 1969, 223.
[17] A. T. Clarke and P. G. Mann, *Biochem. J.*, 1959, **71**, 596; Cl. Schopf, F. Braun, K. Burkhardt, G. Dummer, and H. Muller, *Annalen*, 1959, **626**, 1959.

cule of tetrahydropyridine (III) yields anaferine (VIII), while cyclization of *N*-methylisopelletierine yields pseudopelletierine (IX).

This scheme precludes the involvement of a symmetrical intermediate, such as cadaverine, in the biosynthesis of these alkaloids. Recently, however, it has been reported that [1,5-^{14}C]cadaverine is incorporated into *N*-methylisopelletierine in *P. granatum*.[15]



SCHEME 1

The incorporation of [2-^{14}C]lysine into carbon-2 of this alkaloid in *P. granatum* now reported establishes cadaverine as a compound which, while not a normal intermediate in the biosynthetic pathway, can be metabolised by the plant to a normal intermediate, presumably 2,3,4,5-tetrahydropyridine. This finding is in agreement with the role of cadaverine in the biosynthesis of anabasine.[18]

To substantiate the acetate origin of the three-carbon unit in isopelletierine, methylisopelletierine, pseudopelletierine, and anaferine, sodium [1-^{14}C]acetate (total activity $2{\cdot}2 \times 10^8$ counts/min.) was fed in separate experiments by the wick method to three two-year old *P. granatum* plants and six one-year old, *W. somnifera* plants. The alkaloids from *P. granatum* were isolated and separated as outlined in the Experimental section. On purification to constant activity they had the following specific activities: isopelletierine $6{\cdot}51 \times 10^4$ counts min.$^{-1}$ mmole^{-1} (incorporation 0·021%), methylisopelletierine $8{\cdot}9 \times 10^4$ counts min.$^{-1}$ mmole^{-1} (0·024%), pseudopelletierine $6{\cdot}92 \times 10^4$ counts min.$^{-1}$ mmole^{-1} (0·020%). Anaferine from *W. somnifera* was isolated by established methods and purified to constant activity (specific activity $1{\cdot}08 \times 10^5$, incorporation 0·01%).

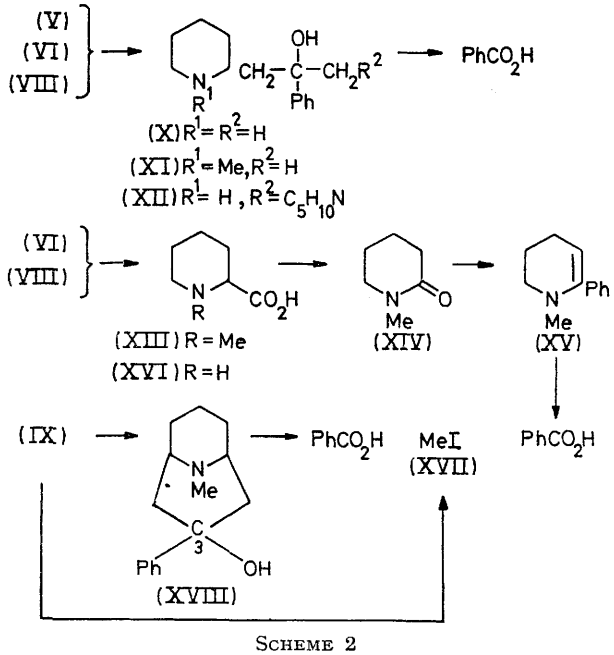
The alkaloids, in turn, were treated with phenylmagnesium bromide to yield the corresponding carbinols (X), (XI), and (XII) which, on oxidation with chromium trioxide–sulphuric acid afforded benzoic acid. The specific activities of the alkaloids and their degradation products are reported in Table 1. The

TABLE 1

Activities of isopelletierine, methylisopelletierine, anaferine, and their degradation products from sodium [1-^{14}C] acetate feed (counts min.$^{-1}$ mmole^{-1} $\times$ 10^{-4})

Methylisopelletierine	8·9
1-(*N*-Methyl-2-piperidyl)-2-phenylpropan-2-ol	8·71
Benzoic acid	8·74
Isopelletierine	6·51
2-Phenyl-1-(2-piperidyl)propan-2-ol	6·30
Benzoic acid	6·24
Psuedopelletierine	6·92
9-Methyl-3-phenyl-9-azabicyclo[3,3,1]nonan-3-ol	6·61
Benzoic acid	6·70
Anaferine	6·53
2-Phenyl-1,3-di-(2-piperidyl)propan-2-ol	6·37
Benzoic acid	6·56

concordance between the specific activities of isopelletierine, methylisopelletierine, pseudopelletierine, and anaferine and their degradation products proves that these alkaloids were labelled only on the starred carbon atoms in (V), (VI), (VIII), and (IX). In a separate experiment sodium [2-^{14}C]acetate was fed in a similar manner



SCHEME 2

to three two-year old *P. granatum* plants, and *N*-methylisopelletierine was isolated. The 9-carbon atom was isolated as iodoform and found to contain one half of the total activity of the methylisopelletierine. These

[18] L. C. Craig, *J. Amer. Chem. Soc.*, 1933, **55**, 295.

results establish the acetate origin of the three-carbon fragment of all these alkaloids.

In a second series of experiments we investigated the role of lysine as a precursor of the piperidine nucleus in two of these alkaloids, namely methylisopelletierine and anaferine. DL-[2-^{14}C]Lysine was fed to three two-year old *P. granatum* and six six-month old *W. somnifera* plants, and *N*-methylisopelletierine and anaferine were isolated as already described. The alkaloids were purified to constant activity and had the following specific activities: *N*-methylisopelletierine $8{\cdot}18 \times 10^4$ counts min.$^{-1}$ mmole^{-1} (incorporation 0·010%), anaferine $1{\cdot}08 \times 10^{-5}$ counts min.$^{-1}$ mmole^{-1} (0·012%).

The methylisopelletierine, on oxidation with chromium trioxide–sulphuric acid, yielded *N*-methylpiperidine-2-carboxylic acid (XIII) which, on refluxing in ethanol saturated with dry hydrogen chloride, afforded the corresponding ethyl ester. The ester was subjected to a modified Barbier-Wieland degradation, which yielded *N*-methyl-2-piperidone (XIV). Reaction of the piperidone with phenylmagnesium bromide, followed by oxidation of the resulting 1,2,3,4-tetrahydro-*N*-methyl-6-phenylpyridine (XV) gave benzoic acid.

The anaferine was oxidised to piperidine-2-carboxylic acid (XVI) with chromium trioxide–sulphuric acid. The amino acid was readily *N*-methylated with formaldehyde–formic acid and the product was degraded as before.

The specific activities of methylisopelletierine, anaferine, and their degradation products are reported in Table 2. It was found that in both anaferine and

TABLE 2

Activities of methylisopelletierine, anaferine, and their degradation products from lysine feed (counts min.$^{-1}$ mmole^{-1} $\times$ 10^{-4})

Methylisopelletierine	8·18
N-Methylpyridine-2-carboxylic acid	7·85
1,2,3,4-Tetrahydro-*N*-methyl-6-phenylpyridine	7·90
Benzoic acid	7·93
Anaferine	10·8
Pyridine-2-carboxylic acid	5·3
1,2,3,4-Tetrahydro-*N*-methyl-6-phenylpyridine	5·4
Benzoic acid	5·5

methylisopelletierine the activity was confined to the marked (■) carbon atoms in (VI) and (VIII). These results substantiate the role of lysine in the biosynthesis of the piperidine nucleus of these alkaloids and show that lysine is incorporated as in the case of anabasine, sedamine, and lobinaline, *via* an unsymmetrical intermediate.

The conversion of *N*-methylisopelletierine into pseudopelletierine was investigated by feeding [N-*methyl*-^{14}C, 8-^{14}C]methylisopelletierine to two two-year old *P. granatum* plants. The plants were harvested after 7 days and after the addition of inactive pseudopelletierine (100 mg.), to serve as carrier, radioactive pseudopelletierine was isolated. It was purified to constant activity (specific activity $4{\cdot}22 \times 10^5$ counts min.$^{-1}$ mmole^{-1}, incorporation 1·2%).

The active pseudopelletierine was subjected to a Hertzig–Meyer reaction, the methyl iodide (XVII) being isolated as triethylmethylammonium iodide. Treatment of the pseudopelletierine with phenylmagnesium bromide yielded the carbinol (XVIII) which, on oxidation, yielded benzoic acid. As a result of this degradation the pseudopelletierine was found to have the same ratio of activity at *N*-methyl to activity at C-3, as the administered methylisopelletierine. The specific activities of the pseudopelletierine and its degradation products are listed in Table 3. This result

TABLE 3

Activity of pseudopelletierine and its degradation products from double-labelling experiment (counts min.$^{-1}$ mmole^{-1} $\times$ 10^{-5})

Pseudopelletierine	4·42
Benzoic acid	0·96
Triethylmethylammonium iodide	3·26
Ratio of activity at *N*-methyl to activity at C-3	3·4 : 1

establishes that methylisopelletierine is incorporated into pseudopelletierine without degradation.

In a further experiment [8-^{14}C]isopelletierine was administered to six three-month old, *W. somnifera* plants as before. The plants were harvested after 10 days and labelled anaferine was isolated; inactive anaferine (100 mg.) being added as carrier. The anaferine was purified to constant activity (specific activity $3{\cdot}5 \times 10^5$ counts min.$^{-1}$ mmole^{-1}, incorporation 2·1%).

The active anaferine was degraded as before to the carbinol (XII) and benzoic acid. The specific activities of anaferine and its degradation products are shown in Table 4.

TABLE 4

Activity of anaferine and its degradation products from isopelletierine feed (counts min.$^{-1}$ mmole^{-1} $\times$ 10^{-5})

Anaferine	3·50
2-Phenyl-1,3-di-(2-piperidyl)propan-2-ol	3·47
Benzoic acid	3·48

The high incorporation (2·1%) of isopelletierine into anaferine strongly suggests that isopelletierine is incorporated directly into anaferine.

EXPERIMENTAL

M.p.s are corrected. Radioactive assays were carried out with a Nuclear-Chicago model D-47 gas-flow counter, with a Micromil window, on samples of finite thickness with appropriate corrections for efficiency and self-absorption. The ethyl [3-^{14}C]acetoacetate was purchased from the Radiochemical Centre, Amersham.

Administration of Tracers to P. granatum *and Isolation of Isopelletierine, Methylisopelletierine, and Pseudopelletierine.*—DL-[2-^{14}C]Lysine (total activity 0·1 mc) in water (3 ml.) was fed by the wick arrangement to three two-year old *P. granatum* plants growing in soil in a greenhouse. The plants were allowed to flag slightly before feeding. The tracer solution had been absorbed by the plants after 24 hr. Water (3 ml.) was fed to each plant through the wick for 2

days. The plants were then grown-on in the normal manner and harvested after a further 12 days. They were homogenised in a Waring blendor with a mixture (1 : 1) of 95% ethanol and concentrated hydrochloric acid (5 ml.); the mixture was kept cool by the addition of solid carbon dioxide. After the addition of methylisopelletierine hydrochloride [19] (100 mg.) as carrier, the mixture was kept at 4° for 3 days and filtered through cloth, and the plant residue was washed with ethanol. The combined washings and filtrate were concentrated to *ca.* 10 ml., water (10 ml.) was added, and the aqueous solution was washed with chloroform–ether (1 : 1) to remove non-basic material. After basification with 20% sodium hydroxide, the solution was extracted with chloroform. Evaporation of the dried (Na_2SO_4) extract yielded a yellow oil.

T.l.c. analysis of this oil on silica gel with chloroform–methanol–diethylamine (90 : 5 : 5) showed the presence of methylisopelletierine (R_F 0·43) and pseudopelletierine (R_F 0·54).

The alkaloid mixture was dissolved in ethanol saturated with dry hydrogen chloride and a little ether was added. The mixture, when left overnight, deposited a white solid which yielded methylisopelletierine hydrochloride (102 mg.), m.p. 158—159° (from ethanol–ether).

In a second experiment sodium [1-^{14}C]acetate (total activity 0·2 mc) was fed in a like manner to three more *Punica* plants. After the addition of inactive methylisopelletierine as carrier, the active methylisopelletierine hydrochloride was isolated as before.

In a third experiment sodium [2-^{14}C]acetate was fed to three *Punica* plants and radioactive methylisopelletierine was again isolated.

In a fourth experiment sodium [1-^{14}C]acetate was fed to *P. granatum*; isopelletierine and pseudopelletierine were isolated and found to be radioactive.

Degradation of Methylisopelletierine.—(a) *Sodium* [1-^{14}C]-*acetate feed.* 1-(N-*Methyl*-2-*piperidyl*)-2-*phenylpropan*-2-*ol.* The active methylisopelletierine (50 mg.) in absolute ether (4 ml.) was added to a two-fold excess of phenylmagnesium bromide. The solution was stirred under nitrogen at room temperature for 24 hr. The magnesium complex was hydrolysed with a cold saturated solution of ammonium chloride, and the mixture was extracted with ether. Evaporation of the ether yielded a viscous oil which gave the *carbinol* (61 mg.), m.p. 114—115° (from ether) (Found: C, 76·8; H, 10·3; N, 5·8. $C_{15}H_{24}NO$ requires C, 76·85; H, 10·3; N, 5·9%).

Benzoic acid. The carbinol (50 mg.) was refluxed with a 10% solution of potassium permanganate (100 ml.) for 4 hr. The mixture was cooled, ethanol (2 ml.) was added to decompose the excess of permanganate, and the solution was extracted with ether. Evaporation gave benzoic acid, which was purified by crystallisation from water and sublimation (10 mg.), m.p. 120—121°.

(b) *Sodium* [2-^{14}C]*acetate feed.* The active methylisopelletierine hydrochloride (60 mg.) was dissolved in 2N-sodium hydroxide and treated with a solution of iodine (0·12 g.) and potassium iodide (0·2 g.) in water (5 ml.). The mixture was stirred for 20 min. and the precipitated iodoform was filtered off, m.p. 120°.

(c) *Lysine feed.* The active methylisopelletierine was diluted with inactive alkaloid to give a total weight of 500 mg.

N-*Methylpiperidine*-2-*carboxylic acid.* Methylisopelletierine hydrochloride (500 mg.) was dissolved in sulphuric acid (20%; 25 ml.) and chromium trioxide (1 g.) in water (35 ml.) was added. The mixture was heated at 90° for 4 hr., then cooled to room temperature; water (10 ml.) was added, and the solution was extracted with ether. This extract was inactive and was discarded.

Sulphur dioxide was passed through the solution, which was then heated to remove excess of the gas. Sulphate ion was removed by the dropwise addition of a saturated solution of barium hydroxide. Barium sulphate was filtered off, and the filtrate, after neutralisation with ammonium hydroxide, was concentrated to small volume. This solution was applied to a column (2 × 30 cm.) of Amberlite IR 120 (H^+). The column was washed with water (200 ml.) and eluted with 1% ammonium hydroxide. Evaporation of the eluate gave a white solid which yielded *N*-methylpiperidine-2-carboxylic acid (246 mg.), m.p. 214—216° (from ethanol–ether) (Found: C, 58·75; H, 9·0; N, 9·6. Calc. for $C_7H_{13}NO_2$: C, 58·7; H, 9·15; N, 9·8%).

Ethyl N-*methylpiperidine*-2-*carboxylate.* The amino-acid (220 mg.) was dissolved in absolute ethanol (10 ml.) saturated with dry hydrogen chloride and the mixture was refluxed for 4 hr. It was then cooled and concentrated; water (10 ml.) was added, and the solution was neutralised with 5% sodium carbonate and extracted with ether. Evaporation of the dried (Na_2SO_4) extract yielded the ethyl ester, which was identical (i.r. spectrum) with an authentic sample.

N-*Methyl*-2-*piperidone.* The ethyl ester (200 mg.) in ether (8 ml.) was added to a solution of phenylmagnesium bromide at −30°. The mixture was stirred under nitrogen and at room temperature for 24 hr. The magnesium complex was hydrolysed with cold 1% hydrochloric acid. The aqueous layer was separated, washed with ether, basified with dilute sodium hydroxide, and extracted with chloroform to yield the carbinol, which was not purified further but was immediately dehydrated by refluxing for 1 hr. with a mixture of acetic acid (15 ml.) and acetic anhydride (15 ml.). Evaporation left a brown residue, which was dissolved in ether (5 ml.) and pyridine (0·3 ml.). The solution was cooled to −40°, osmium tetroxide (100 mg.) in ether was added, and the mixture was stirred for 40 min. It was then allowed to come to room temperature and the osmate ester–pyridine complex was filtered off and added to a solution of sodium sulphite (260 mg.) and potassium carbonate (80 mg.) in 50% ethanol (12 ml.). After being stirred for 1 hr. the solution was extracted with ether to yield the glycol as a viscous oil which, on treatment with sodium periodate (150 mg.) in water (30 ml.), yielded *N*-methyl-2-piperidone, identical (i.r. spectrum and m.p. of picrate) with an authentic sample.

1,2,3,4-*Tetrahydro*-N-*methyl*-6-*phenylpyridine.* The piperidone (40 mg.) in ether was added to an excess of phenylmagnesium bromide. The product (picrate m.p. 144—145°) was isolated by the method of Craig.[18]

Benzoic acid. The tetrahydro-*N*-methyl-6-phenylpyridine (40 mg.) was refluxed for 2 hr. in a 1% aqueous solution (100 ml.) of potassium permanganate. Ethyl alcohol was added to decompose the excess of permanganate, the mixture was filtered, and the benzoic acid was isolated by extraction with ether (9 mg.), m.p. 120—121° (from water).

[19] Cl. Schöpf, A. Komaz, Fr. Braun, and E. Jacobe, *Annalen*, 1948, **559**, 1.

Administration of Labelled Methylisopelletierine to P. granatum *and Isolation of Pseudopelletierine.*—(±)-[N-*methyl*-^{14}C, 8-^{14}C]Methylisopelletierine (3 mg.; total activity $2{\cdot}3 \times 10^7$ counts min.$^{-1}$; ratio of activity at *N*-methyl to activity at C-8 3·6 : 1) was administered by the wick method to two two-year old *P. granatum* plants. After 7 days, the plants were harvested and homogenised in a Waring blendor with a mixture (1 : 1; 5 ml.) of 95% ethanol and concentrated hydrochloric acid (5 ml.), with inactive pseudopelletierine (100 mg.) as carrier. After 3 days at 0° the mixture was filtered and the filtrate was concentrated. The residue was dissolved in water and the mixture was washed with chloroform–ether (1 : 1) to remove non-basic material. The aqueous solution was basified and extracted with chloroform to yield a yellow solid, which, on crystallisation from pentane followed by sublimation (40°/0·3 mm.), gave pseudopelletierine (98 mg.), m.p. 63—64°.

Degradation of the Pseudopelletierine.—The active pseudopelletierine (40 mg.) was dissolved in ether (10 ml.) and added to an excess of phenylmagnesium bromide. The mixture was stirred for 24 hr. and the phenyl derivative (52 mg.) was isolated as described for 1-(*N*-methyl-2-piperidyl)-2-phenylpropan-2-ol.

Hertzig–Meyer Reaction of Pseudopelletierine.—This reaction was carried out as previously described;[16] the methyl iodide was isolated as triethylmethylammonium iodide, m.p. 296—297° (from propan-2-ol).

Administration of Tracers to W. somnifera *and Isolation of Anaferine.*—(±)-[2-^{14}C]-Lysine (14 mg.; total activity $5{\cdot}76 \times 10^6$ counts min.$^{-1}$) and sodium [1-^{14}C]acetate (4 mg.; total activity $2{\cdot}2 \times 10^8$ counts min.$^{-1}$) were administered, in separate experiments, to six six-month old *W. somnifera* plants growing in an aerated inorganic nutrient solution.[9] In both experiments, over 90% of the activity had been absorbed by the plants after 36 hr.; uptake of tracer was followed by measuring the loss of activity from the solution. After a further 24 hr., the plants were harvested and homogenised in a Waring blendor with a mixture (1 : 1) of ethanol and concentrated hydrochloric acid (5 ml.). Inactive anaferine hydrochloride (100 mg.) was added as carrier and the mixture was left at 4° for 48 hr. It was then worked up as described for the isolation of methylisopelletierine, and yielded, in both experiments, radioactive anaferine.

In a third experiment (±)-[8-^{14}C]isopelletierine (2 mg.; total activity $5{\cdot}76 \times 10^6$ counts min.$^{-1}$) in 0·01N-hydrochloric acid was administered by the wick method to six three-month old *W. somnifera* plants. The solution had been absorbed by the plants after 24 hr. The plants were watered through the wick for 2 days and were then grown normally. After 8 days, they were harvested and radioactive anaferine was isolated as before.

Degradation of Anaferine.—(a) *Acetate feed. 2-Phenyl-1,3-di-(2-piperidyl)propan-2-ol.* Anaferine (60 mg.) was treated with phenylmagnesium bromide and the resulting magnesium complex was hydrolysed with ice-cold dilute ammonium chloride solution. The carbinol (50 mg.) was refluxed in a 1% solution of potassium permanganate (200 ml.) for 3 hr., then cooled and ethanol was added dropwise to decompose excess of permanganate. The filtered solution was acidified with 10% hydrochloric acid and extracted with ether. Evaporation of the dried (Na_2SO_4) extract yielded benzoic acid, which was purified by sublimation (15 mg.), m.p. 120—121°.

(b) *Lysine feed. Piperidine-2-carboxylic acid.* Anaferine hydrochloride (240 mg.) in sulphuric acid (50% v/v; 50 ml.) was added to chromium trioxide (714 mg.) in water (6 ml.) and refluxed gently for 3 hr. The mixture was cooled and the excess of chromium trioxide was decomposed as before with sulphur dioxide. The concentrated filtrate was applied to a column of Amberlite IR-120 (H^+). The column was washed with water and eluted with 3% ammonium hydroxide to yield a yellow solid, which yielded the (±)-acid (120 mg.), m.p. 270—272° (from methanol–ether).

N-*Methylpyridine-2-carboxylic acid.* Pyridine-2-carboxylic acid (100 mg.) was refluxed for 3 hr. with a mixture of formic acid (90%; 5 ml.) and formaldehyde (40% v/v; 5 ml.). Excess of formaldehyde and formic acid were evaporated off under reduced pressure and the resulting oil crystallised from methanol–ether to yield the *N*-methyl acid (104 mg.), identical (i.r. spectrum and mixed m.p.) with an authentic sample.

(c) *Isopelletierine feed.* The anaferine was treated with phenylmagnesium bromide and the resulting carbinol was oxidised to benzoic acid as before.

(±)-[8-^{14}C]*Isopelletierine.*[20] Finely powdered dodecahydro-1*H*,6*H*,11*H*-tripyrido[1,2-*a*:1′,2′-*c*:1″,2″-*e*]-*s*-triazine (83 mg.) was added to a solution of [3-^{14}C]-acetoacetic acid {prepared by dissolving ethyl [3-^{14}C]-acetoacetate (260 mg.; specific activity $5{\cdot}5 \times 10^8$ counts min.$^{-1}$ mmole^{-1}) in a solution of sodium hydroxide (93 mg.) in water (5 ml.) and leaving the solution at 4° for 24 hr.}. The mixture was stirred for 3 hr., after which the terpiperidyl had dissolved and the solution had a pH of 11·5. The solution was left at 20° for a further 20 hr.; the pH was kept constant (±0·5) by addition of 2% sodium hydroxide. After acidification with dilute hydrochloric acid, the solution was extracted with ether, basified with 20% sodium hydroxide, and extracted with chloroform. Evaporation of the dried (Na_2SO_4) extract yielded a colourless oil, which was dissolved in ethanol saturated with dry hydrogen chloride, and ether was added. The mixture deposited a white solid overnight, which yielded isopelletierine hydrochloride (81 mg.), m.p. 145—146° (from ethanol–ether) (specific activity $5{\cdot}1 \times 10^8$ counts min.$^{-1}$ mmole^{-1}, identical (mixed m.p. and i.r. spectrum) with an authentic sample.

(±)-[*N*-methyl-^{14}C]*Methylisopelletierine.* Isopelletierine (80 mg.) was dissolved in absolute ether (20 ml.) and [^{14}C]methyl iodide (70 mg.; specific activity $2{\cdot}2 \times 10^8$ counts min.$^{-1}$ mmole^{-1}) was added. The mixture was stirred at room temperature for 3 days then added to 2N-sodium hydroxide (60 ml.), and the ether was separated. The aqueous solution was extracted with ether (5 × 20 ml.). Evaporation of the dried (Na_2SO_4) extracts yielded a yellow oil which, on a t.l.c. plate (silica gel G) with benzene–diethylamine–methanol (99 : 5 : 1), showed the presence of isopelletierine R_F 0·36), methylisopellerine (R_F 0·41), and an unidentified compound (R_F 0).

The mixture was chromatographed on a column (1 × 10 cm.) of basic alumina (activity I) with chloroform as eluant. The initial fractions were shown to consist of methylisopelletierine. Evaporation of the chloroform gave a colourless oil which was converted into the hydrochloride as described for isopelletierine and crystallised to

[20] F. Galinovosky, A. Wagner. and R. Eeiser, *Montash.*, 1951, **82**, 551.

constant activity (20 mg.), m.p. 157—158° (specific activity $1{\cdot}89 \times 10^9$ counts min.$^{-1}$ mmole^{-1}).

(±)-[8-^{14}C]-N-*Methylisopelletierine.* [3-^{14}C]Acetoacetic acid, prepared as before from the ester (260 mg.; specific activity $5{\cdot}5 \times 10^8$ counts min.$^{-1}$ mmole^{-1}) was treated with *N*-methylpiperidin-2-ol in ether [prepared from *N*-methyl-2-piperidone (210 mg.) as described by Galinovosky [19]]. The ether was evaporated off and the aqueous solution, adjusted to pH 7 with 1% hydrochloric acid, was kept at room temperature for 48 hr. Basification followed by extraction with chloroform yielded an oil, which was dissolved in ethanol. The solution was saturated with dry hydrogen chloride and treated with ether. The resulting solid was crystallised from ethanol–ether to give methylisopelletierine hydrochloride (106 mg.; specific activity $5{\cdot}2 \times 10^8$ counts min.$^{-1}$ mmole^{-1}).

Equimolar amounts of the two labelled hydrochlorides were mixed and recrystallised from ethanol–ether to give (±)-[*methyl*-^{14}C, 8-^{14}C]methylisopelletierine hydrochloride (specific activity $1{\cdot}2 \times 10^9$ counts min.$^{-1}$ mmole^{-1}; ratio of activity at *N*-methyl to activity at C-8 3·6 : 1).

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