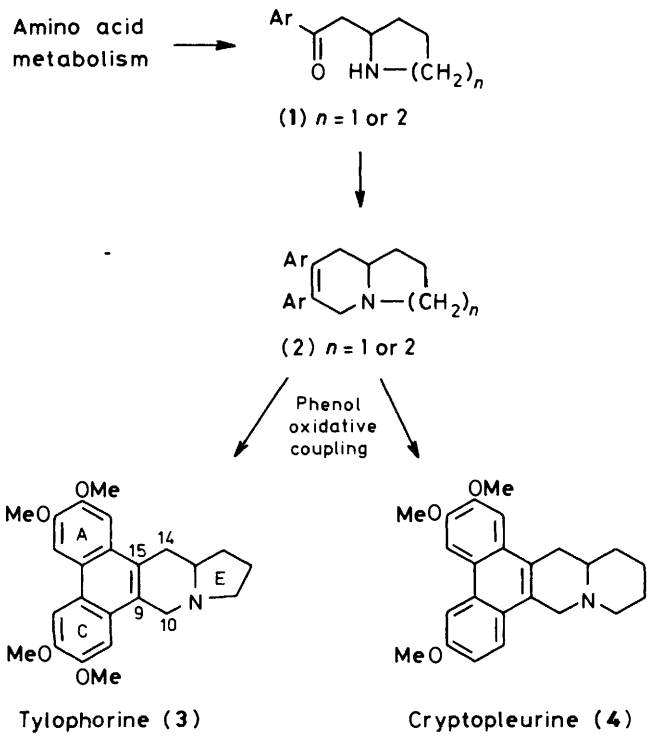
Biosynthesis of Phenanthroindolizidine Alkaloids: Incorporation of 2-Pyrrolidin-2-ylacetophenone and Benzoylacetic Acid and Derivatives†

Richard B. Herbert,* Frederick B. Jackson, and Ian T. Nicolson
Department of Organic Chemistry, The University, Leeds LS2 9JT

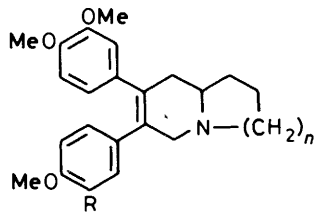
2-Pyrrolidin-2-ylacetophenone (**12**) and its oxygenated derivatives, (**13**) and (**14**), bearing ^{14}C and ^{3}H labels are synthesized and are shown to be intact precursors for the phenanthroindolizidine alkaloid, tylophorinine (**16**), in *Tylophora asthmatica*; the three amines, singly labelled with tritium, are shown to be precursors for tylophorine (**3**) and tylophorinidine (**17**). Benzoylacetic acid (**9**) and *p*-hydroxybenzoylacetic acid (**10**), but not 4-hydroxy-3-methoxybenzoylacetic acid, are also precursors for tylophorinine (**16**). The results allow partial description of the biosynthetic pathways to phenanthroindolizidine alkaloids. A degradation is described which allowed the location of label in 2-pyrrolidin-2-ylacetophenone, derived from [5-^{14}C]ornithine, to be established.

Tylophorine (**3**) and cryptopleurine (**4**) are representatives of a family of phenanthroindolizidine and phenanthroquinolizidine alkaloids. A reasonable hypothesis (Scheme 1) may be derived for the biosynthesis of these alkaloids which is based in part on the known natural occurrence of the following: (*a*) seco-bases, *e.g.* (**5**)[1] and (**6**);[2] (*b*) 2-(2-piperidyl)acetophenones, *e.g.* (**7**),[3]

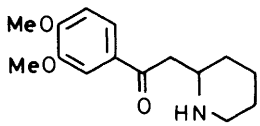


Scheme 1.

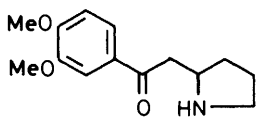
and (*post facto*) the alkaloids (**8**) and (**14**) of *Ruspolia hypercrateriformis* which have been discovered recently.[4] 2-(2-Piperidyl)acetophenones and 2-pyrrolidin-2-ylacetophenones [as (**1**)] stand as pivotal intermediates in the biosynthetic hypothesis (Scheme 1, see also Scheme 2). Examination of these compounds as precursors provides a crucial test for the correctness of the hypothesis and results reported here establish that 2-pyrrolidin-2-ylacetophenone (**12**) and its derivatives (**13**)



(**5**) R = OMe, *n* = 1
(**6**) R = H, *n* = 2



(**7**)



(**8**)

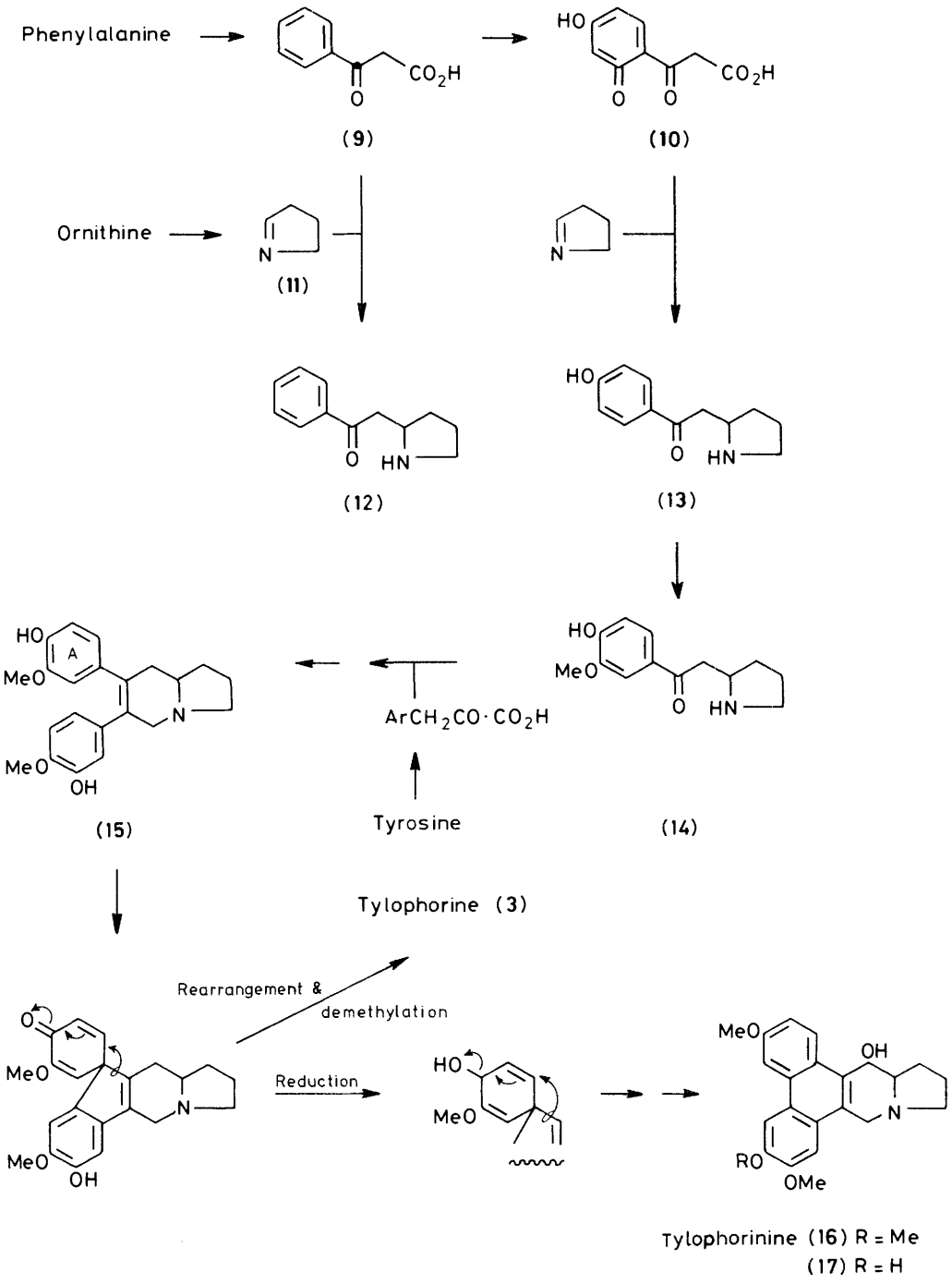
and (**14**) are intact precursors for tylophorinine (**16**) in *Tylophora asthmatica* Wight et Arn.

Evidence presented elsewhere relates to the intermediacy of compounds of type (**15**) in the biosynthesis of phenanthroindolizidine alkaloids.[5,6] The origins of this skeleton in tylophorine (**3**) have been shown to be in phenylalanine[7] *via* cinnamic acid[8] (ring A plus C-14 and C-15), and, independently, in tyrosine[9] *via* dopa[6] (ring C plus C-9 and C-10). Preliminary results indicate that ornithine, as expected, provides the nitrogen atom and the remaining carbon atoms of the tylophorine skeleton (ring E).[7,10] The known pathways[11] to a number of alkaloids based on the unit (**1**) and these results are together consistent with the derivation of phenanthroindolizidine alkaloids *via* 2-pyrrolidin-2-ylacetophenones [as (**12**)].

2-Pyrrolidin-2-ylacetophenone (**12**) is conveniently prepared by condensation of benzoylacetic acid (**9**) with 3,4-dihydro-5*H*-pyrrole (1-pyrroline) (**11**).[12] 3,4-Dihydro-5*H*-pyrrole is simply prepared either by reaction of ornithine (**18**) with *N*-bromosuccinimide or by reaction of putrescine (**19**) with the enzyme, diamine oxidase, isolated from pea seedlings; the latter method gave better yields of (**12**), but the former was used in all experiments described here. The commercial availability of putrescine and ornithine with various radioactive labels meant that suitably labelled samples of (**12**) and related compounds were easily accessible.

Tylophorine (**3**) and tylophorinine (**16**) are both produced by *T. asthmatica*. A detailed consideration of the late stages of the

† Preliminary communication: R. B. Herbert, F. B. Jackson, and I. T. Nicolson, *J. Chem. Soc., Chem. Commun.*, 1976, 865.



Scheme 2.

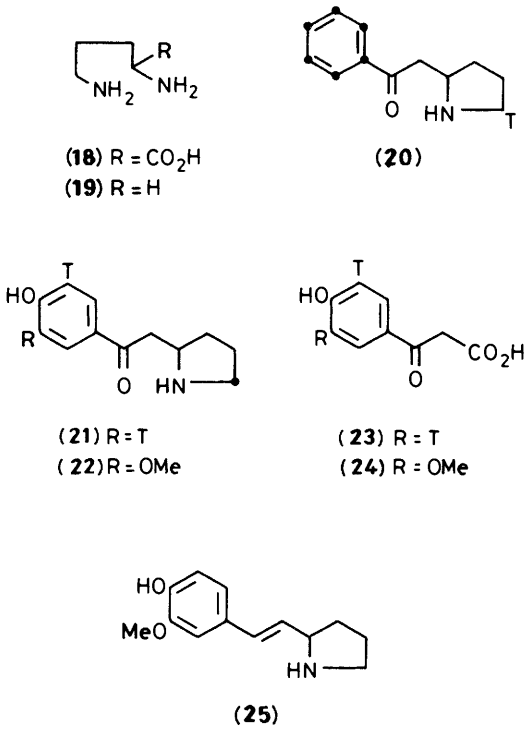
biosynthesis of these alkaloids indicated that they might both be formed *via* the diarylhexahydroindolizine (**15**). Phenol oxidative coupling within (**15**) and following orthodox reactions would give (**3**) and (**16**) (Scheme 2) (subsequently shown to be correct).[5,6] The necessary hydroxylation and methylation required for the substitution pattern seen in ring A of (**15**) could be introduced either before, or after, indolizine formation. This was tested for by examining 4′-hydroxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone (**14**) and 4′-hydroxy-2-pyrrolidin-2-ylacetophenone (**13**) as precursors.

The putative precursors, (**13**) and (**14**) were synthesized in a similar way to (**12**); phenolic functions were protected during the syntheses as benzyl ethers, the protecting group being removed by hydrogenolysis in the ultimate step. Intact utilization of each of the precursors, (**12**), (**13**), and (**14**), was monitored by the presence in each precursor of tritium and ^{14}C labels which were well separated within the skeleton and were of known ratio. Tritium adjacent to phenolic functions in (**13**) and (**14**) was introduced by an excellent exchange procedure.[13] The sites of labelling were checked by synthesizing and analysing the corresponding deuteriated compounds. Further checking showed that deuterium (tritium) was not removed during the synthetic sequence. The radioactive compounds used for the biosynthetic experiments were (**20**), (**21**), and (**22**) (^{14}C: ●).

Table. Incorporation of precursors into tylophorinine (**16**)[a]

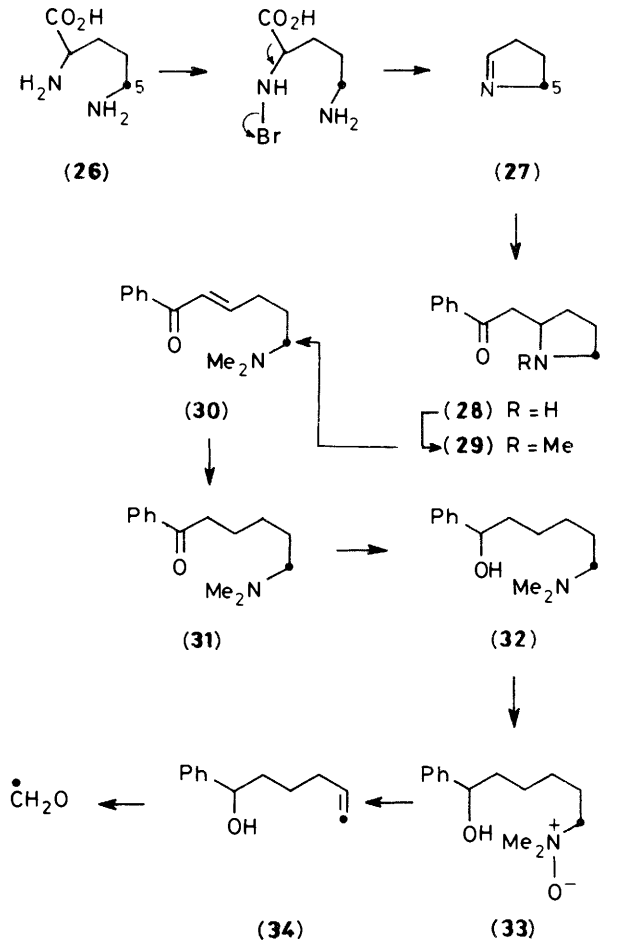
Precursor	Administered $^3H/^{14}C$	Isolated $^3H/^{14}C$	Incorporation (%)
(**9**)[b]			1.5×10^{-3}
(**23**)[b]			5.8×10^{-3}
(**24**)[b]			Inactive alkaloid
(**20**)[c]	12.6	13.0	1.7×10^{-1}
(**21**)[b]	6.1	2.6	3.8×10^{-3}
(**22**)[b]	2.7	3.2	3.5×10^{-2}

[a] Isolated as its acetate. [b] Absorption into excised stems. [c] Wick-feed to whole plants.



When the compounds (**20**), (**21**), and (**22**) were administered to *T. asthmatica*, each of them was found to be a satisfactory precursor for tylophorinine (**16**) (Table). The amines (**20**) and (**22**) were incorporated essentially without change in isotope ratio and therefore they were utilized intact for biosynthesis. The incorporation of base (**21**) into tylophorinine (**16**) resulted in loss of essentially half of the tritium label. This was, however, expected since implication of (**21**) in the biosynthetic pathway logically involves entry of a hydroxy group at one of the two tritiated sites. Thus (**21**) was also incorporated intact. These results strongly indicate that the biosynthesis of tylophorinine (**16**) proceeds by way of 2-pyrrolidin-2-ylacetophenones and involves in part the sequence: (**12**) ⟶ (**13**) ⟶ (**14**). In addition, the results point to an indolizine such as (**15**), with a dioxygenated ring A, being involved in the biosynthesis of (**16**).

Further support for, and definition of, the early part of the pathway shown in Scheme 2 comes from the finding that the keto acids (**9**) and (**23**), but not (**24**) (labels as shown; ^{14}C: ●) were satisfactory precursors for tylophorinine (**16**) (Table). Although only a single label was used and the sites of labelling in the derived alkaloid were not established, the observation that there was a marked distinction in the incorporation of label from (**9**) and (**23**) compared to (**24**) and that the levels of



Scheme 3.

incorporation of (**9**) and (**23**) were similar to those of (**21**) and (**22**) argues strongly that these keto acids were incorporated intact. The incorporation of (**23**) indicates that hydroxylation of the aromatic nucleus can occur before formation of the 2-pyrrolidin-2-ylacetophenone skeleton as an alternative to hydroxylation of (**12**). However, the failure of (**24**) to act as a precursor suggests that only a single oxygen function may be introduced at the keto acid level of oxidation. The pattern of biosynthesis deduced from the results is summarized in Scheme 2.

A puzzling feature of the above results was the isolation in all our experiments of inactive samples of tylophorine (**3**) and tylophorinidine (**17**) which are, like (**16**), major alkaloids of *T. asthmatica*. In preliminary studies with tritiated samples of the precursors, samples of (**3**), (**16**), and (**17**) were isolated with similar levels of radioactivity so we presume that at the time of our later experiments neither (**3**) nor (**17**) were being biosynthesized.

The amine (**14**), which the above evidence points to as a key intermediate in the biosynthesis of phenanthroindolizidine alkaloids, is norruspolinone, an alkaloid produced by *R. hypercrateriformis*.[4] The spectral data on our synthetic amine (**14**) are identical with those of natural norruspolinone. Additional evidence is thus provided for the correctness of the structure for the *R. hypercrateriformis* alkaloid. The benzyl ether of (**14**), it may be noted, has been used to synthesize norruspoline (**25**) another alkaloid produced by this plant.[14]

In the reaction of ornithine (**26**) with *N*-bromosuccinimide which gives (**27**)[12,15] it is predictable that C-5 of the amino acid

becomes C-5 of 3,4-dihydro-5*H*-pyrrole (**27**) and of the 2-pyrrolidin-2-ylacetophenone (**28**) derivable from (**27**). We have confirmed this by a degradation of (**28**) formed from [5-^{14}C]ornithine (**26**) (Scheme 3).

The radioactive 2-pyrrolidin-2-ylacetophenone (**28**) was first converted into its *N*-methyl derivative (**29**) by an Eschweiler-Clarke reaction. This reaction went in poor yield as did an alternative using sodium cyanoborohydride reduction[16] of the imine formed between (**28**) and formaldehyde. Radioactive (**29**) was diluted with inactive (**29**) more readily obtained by reducing *N*-methylpyrrolidone with lithium aluminium hydride and treating the resultant *N*-methyldihydropyrrolium salt with benzoylacetic acid.[17]

The methiodide of (**29**) underwent a Hofmann elimination in aqueous sodium hydroxide to give (**30**) which gave (**31**) on hydrogenation. Neither the methiodide of (**31**) nor that of (**32**) (*cf.* ref. 18) successfully underwent Hofmann elimination but the *N*-oxide (**33**) gave the olefin (**34**) on heating (*cf.* ref. 19). The olefin gave formaldehyde, isolated as its dimedone derivative, on treatment with osmium tetraoxide and sodium metaperiodate. The formaldehyde contained all the radioactivity of the starting ornithine.

Experimental

M.p.s were obtained on a hot-stage apparatus. N.m.r. spectra were recorded at 90 MHz on a Perkin-Elmer R32 instrument unless otherwise stated, and mass spectra were obtained on an AEI MS902 mass spectrometer. Unless stated otherwise column chromatography was carried out using Kieselgel.[20] Organic extracts were dried with magnesium sulphate. Throughout, ether refers to diethyl ether. Radioactive compounds were purchased either from Amersham International or New England Nuclear. Radioactivity was measured by scintillation counting on a Packard Tricarb machine; [^{14}C]- and [^{3}H]-hexadecane standards were used as internal standards in each assay. The purity of radioactive precursors was confirmed by t.l.c. followed by autoradiography.

Benzyl 4-Benzyloxy-3-methoxybenzoate.—Vanillic acid (6.0 g, 35.7 mmol) and benzyl chloride (10.1 g, 80 mmol) were dissolved in acetonitrile (100 ml) and stirred with anhydrous potassium carbonate (10 g) and sodium iodide (5 g). The mixture was refluxed overnight and was then cooled. It was filtered and the solid was washed with acetonitrile. The combined solutions were evaporated to give benzyl 4-benzyloxy-3-methoxybenzoate (11.8 g, 95%) which was used for the next reaction without purification. The product could be recrystallized from ethanol and had m.p. 89—90 °C; $\nu_{max.}$(Nujol) 1 708, 1 603, and 1 590 cm^{-1}; δ($CDCl_3$; 60 MHz) 3.86 (3 H, s), 5.15 (2 H, s), 5.31 (2 H, s), 6.86 (1 H, d, *J* 9 Hz), and 7.12—7.8 (12 H, m); *m/z* 348 (M^+), 181, 151, 122, and 91.

4-Benzyloxy-3-methoxybenzoic Acid and its Acid Chloride.—A solution of benzyl 4-benzyloxy-3-methoxybenzoate (11.0 g) in 2M-aqueous sodium hydroxide (50 ml) and ethanol (50 ml) was refluxed for 6 h. The reaction mixture was acidified and extracted with ether. The combined extracts were dried and the solvent was evaporated to give 4-benzyloxy-3-methoxybenzoic acid (7.8 g, 94%). It could be recrystallized from water, m.p. 169—170 °C (lit.,[21] 169 °C); $\nu_{max.}$(Nujol) 2 500br, 1 680, 1 605, and 1 590 cm^{-1}; δ($CDCl_3$; 60 MHz), 3.95 (3 H, s), 5.25 (2 H, s), 6.95 (1 H, d, *J* 9 Hz), 7.23—7.82 (7 H, m), and 9.02 (1 H, br s, exchanged with D_2O); *m/z* 258 (M^+), 212, 105, and 91.

The acid gave an acid chloride with thionyl chloride (4.5 h reflux); m.p. 62—63 °C from light petroleum (lit.,[22] 61—62 °C); $\nu_{max.}$(Nujol) 1 755, 1 591, and 1 582 cm^{-1}.

Ethyl 4-Benzyloxy-3-methoxybenzoylacetate.—This was prepared by a previously published method[23] from the acid chloride above. It had m.p. 64—65 °C (from aqueous ethanol) (lit.,[23] 68—69 °C); $\nu_{max.}$(Nujol) 1 725, 1 670, 1 600, and 1 590 cm^{-1}; δ($CDCl_3$) 1.17 (3 H, t, *J* 7 Hz), 3.88 (5 H, s), 4.17 (2 H, q, *J* 7 Hz), 5.20 (2 H, s), 6.90 (1 H, d, *J* 9.0 Hz), and 7.17—7.65 (7 H, m); *m/z* 328.129 85 (M^+) ($C_{19}H_{20}O_5$ requires *M*, 328.131 06), 298, 256, 241, 237, 213, 151, 122, and 91.

4-Benzyloxy-3-methoxybenzoylacetic Acid.—Ethyl 4-benzyloxy-3-methoxybenzoylacetate was hydrolysed as described[12] for ethyl benzoylacetate (81%). The acid obtained was characterized as 4-benzyloxy-3-methoxybenzoylacetophenone obtained on heating the β-keto acid with M-sulphuric acid (78%); m.p. 84—86 °C (from aqueous ethanol) (lit.,[24] 85—87 °C); $\nu_{max.}$(Nujol) 1 668, 1 590, and 1 582 cm^{-1}; $\lambda_{max.}$(EtOH) (log ε) 231 (4.01), 275 (3.93), and 306 nm (3.79); δ($CDCl_3$) 2.53 (3 H, s), 3.92 (3 H, s), 5.21 (2 H, s), 6.90 (1 H, d, *J* 9 Hz), and 7.20—7.70 (7 H, m); *m/z* 256.109 93 (M^+) ($C_{16}H_{16}O_3$ requires *M*, 256.109 94), 241, 165, 107, and 91.

4-Hydroxy-3-methoxybenzoylacetic Acid.—A solution of 4-benzyloxy-3-methoxybenzoylacetic acid (69 mg) in dry ethanol (10 ml) together with 10% palladium on charcoal (25 mg) was shaken under hydrogen at atmospheric pressure until no more hydrogen was taken up. The solution was filtered (Celite) and evaporated in the cold. The colourless oil was taken up in aqueous sodium hydrogencarbonate. The solution was washed with ether, cooled in ice, and acidified with M-sulphuric acid. The solution was extracted with ether. The ether extracts were dried and evaporated in the cold to give 4-hydroxy-3-methoxybenzoylacetic acid [as (**24**)] (45 mg, 91%). This was characterized as 4-hydroxy-3-methoxyacetophenone which was obtained on heating it with M-sulphuric acid (66%). The acetophenone had m.p. 113—114 °C (from ethanol) (lit.,[25] 115 °C); $\nu_{max.}$($CHCl_3$) 3 530, 1 670, and 1 593 cm^{-1}; $\lambda_{max.}$(EtOH) (log ε) 231 (4.27), 276 (4.01), and 305 nm (3.93); $\lambda_{max.}$(EtOH + 2M-NaOH) 250 and 347 nm; δ($CDCl_3$) 2.56 (3 H, s), 3.94 (3 H, s), 5.0—5.80 (1 H, br s, exchanged with D_2O), 6.97 (1 H, d, *J* 9 Hz), and 7.48—7.60 (2 H, m); *m/z* 166.062 46 (M^+) ($C_9H_{10}O_3$ requires *M*, 166.062 99).

4′-Benzyloxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone (cf. *Ref.* 12).—Freshly prepared 4-benzyloxy-3-methoxybenzoylacetic acid (320 mg, 1.07 mmol) was dissolved in methanol (15 ml) and phosphate buffer (1.5 ml, 1M, pH 7.25) was added followed by a freshly prepared solution of 3,4-dihydro-5*H*-pyrrole (1-pyrroline)[12,15] (1.0 mmol). The pH was adjusted to 7.0 (1M-KOH) and the reaction mixture was stirred under nitrogen for 60 h at room temperature. The 4′-benzyloxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone (160 mg, 46%) was isolated and purified as described for 2-pyrrolidin-2-ylacetophenone.[12] It had the following spectral characteristics: $\nu_{max.}$(film) 3 360, 1 715, 1 680, and 1 600 cm^{-1}; δ($CDCl_3$; 60 MHz) 1.5—2.1 (4 H, unresolved), 2.1—2.4 (1 H, m, NH), 2.7—3.2 (4 H, unresolved), 3.2—3.65 (1 H, m), 3.95 (3 H, s), 5.2 (2 H, s), 6.7—7.0 (1 H, m), and 7.2—7.7 (7 H, m). It gave an *N*-acetyl derivative (acetic anhydride, pyridine, 4 h reflux) purified by preparative t.l.c. (2% MeOH in $CHCl_3$). The *N-acetyl-4′-benzyloxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone* had the following spectral characteristics: $\nu_{max.}$($CHCl_3$) 1 665, 1 622, and 1 592 cm^{-1}; δ($CDCl_3$) 1.8—2.1 (4 H, unresolved) 2.06 (3 H, s), 2.3—4.0 (4 H, unresolved), 3.97 (3 H, s), 4.3—4.7 (1 H, m), 5.22 (2 H, s), 6.95 (1 H, d, *J* 9 Hz), and 7.2—7.9 (7 H, m); *m/z* 367.178 12 (M^+) ($C_{22}H_{25}NO_4$ requires *M*, 267.178 35), 324, 276, 241, 126, 112, and 91.

4′-Hydroxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone (**14**) [±)-*Norruspolinone*].—A solution of 4′-benzoyloxy-3′-

methoxy-2-pyrrolidin-2-ylacetophenone (70 mg) in ethanol (10 ml) was hydrogenated in the presence of 10% palladium on charcoal (20 mg) at room temperature and atmospheric pressure until hydrogen uptake ceased (6 h). The reaction mixture was filtered (Celite) and the solvent evaporated from the filtrate. The brown oil obtained was purified by preparative t.l.c. (1 part concentrated aqueous ammonia:50 parts methanol:49 parts chloroform). 4′-Hydroxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone (**14**) was isolated as a pale brown gum (91%) which was crystallized from methanol–chloroform as white needles, m.p. 174—176 °C (lit.,[4] 175 °C); $\nu_{max.}$(KBr) 3 550, 3 360, 1 643, 1 595, 1 573, 1 505, 1 332, and 1 120 cm^{-1}; $\lambda_{max.}$(EtOH) (log ε) 228 (4.05), 274 (3.91), 301 (2.87), and 346 (3.21); $\lambda_{min.}$ 215, 244, and 289 nm; $\lambda_{max.}$(EtOH + 2M-NaOH) (log ε) 209 (4.39), 246 (3.87), 302infl. (3.59), and 345 (4.32); $\lambda_{min.}$ 226 and 267 nm; δ($CDCl_3$) 7.2—7.45 (2 H, multiplet but d, *J* 2 Hz, visible at δ 7.41), 6.77 (1 H, d, *J* 8 Hz), 3.84 (3 H, s), 3.4—3.8 (1 H, m), 2.8—3.2 (4 H, unresolved), 3.17 (2 H, s, exchanged with D_2O), and 1.3—2.2 (4 H, unresolved); *m/z* 235.121 40 (M^+) ($C_{13}H_{17}NO_3$ requires *M*, 235.120 84) 166, 151, 123, 108, 84, and 70. Direct comparison of the spectra for this material with those for natural norruspolinone,[4] showed that the two compounds were identical. Compound (**14**) was further characterized as its *N,O*-diacetyl derivative: $\nu_{max.}$($CHCl_3$) 1 768, 1 678, 1 628, and 1 600 cm^{-1}; δ($CDCl_3$) 7.7—7.85 (2 H, m), 7.12 (1 H, d, *J* 8.7 Hz), 4.37—4.67 (1 H, m), 3.93 (3 H, s), 3.86 (1 H, dd, *J* 14 and 8 Hz), 3.3—3.7 (2 H, unresolved), 2.64 (1 H, dd, *J* 14 and 10.5 Hz), 2.33 (3 H, s), 2.07 (3 H, s), and 1.75—2.2 (4 H, unresolved); *m/z* 319.141 35 (M^+) ($C_{17}H_{21}NO_5$ requires *M*, 319.141 96), 277, 276, 234, 151, 126, 112, 84, and 70. Direct comparison of the spectra obtained for this derivative with those of the *N,O*-diacetyl derivative of natural norruspolinone showed that the two compounds were identical.

4-Benzyloxybenzoyl Chloride.—Benzyl 4-benzyloxybenzoate was prepared in 75% yield from 4-hydroxybenzoic acid as described above for benzyl 4-benzyloxy-3-methoxybenzoate; m.p. 119—120 °C (from ethanol) (lit.,[26] m.p. 115.5—116.5 °C); $\nu_{max.}$(Nujol) 1 701 and 1 608 cm^{-1}; δ($CDCl_3$) 5.09 (2 H, s), 5.32 (2 H, s), 6.98 (2 H, d, *J* 9 Hz), 7.1—7.6 (10 H, br s), and 8.03 (2 H, d, *J* 9 Hz); *m/z* 318, 227, 211, 107, and 91. Benzyl 4-benzyloxybenzoate was hydrolysed to 4-benzyloxybenzoic acid (78%) as described above for 4-benzyloxy-3-methoxybenzoic acid, m.p. 186—187 °C (from aqueous ethanol) (lit.,[21] 188 °C); $\nu_{max.}$(Nujol) 2 800—2 400, 1 687, 1 612, and 1 583 cm^{-1}; δ[$(CD_3)_2CO$–$(CD_3)_2SO$; 60 MHz] 5.24 (2 H, s), 5.48—6.42 (1 H, exchanged with D_2O), 7.08 (2 H, d, *J* 9 Hz), 7.23—7.60 (5 H, m) and 7.98 (2 H, d, *J* 9 Hz); *m/z* 228 (M^+) 183, 159, 121, and 91. 4-Benzyloxybenzoyl chloride was prepared in 77% yield as described for 4-benzyloxy-3-methoxybenzoyl chloride above; m.p. 104—106 °C (from light petroleum) (lit.,[21] 106 °C); $\nu_{max.}$(Nujol) 1 770 and 1 598 cm^{-1}.

Ethyl 4-Benzyloxybenzoylacetate.—*Ethyl 2-(4′-benzyloxybenzoyl)acetoacetate* was prepared in 86% yield from 4-benzyloxybenzoyl chloride as described above for ethyl 2-(4′-benzyloxy-3′-methoxybenzoyl)acetoacetate; m.p. 54—56 °C (from aqueous ethanol); *m/z* 340.130 43 (M^+) ($C_{20}H_{20}O_5$ requires *M*, 340.131 06), 298, 211, 183, 121, 105, and 91. This product was converted into *ethyl 4-benzyloxybenzoylacetate* in 44% yield as described above for ethyl 4-benzoyloxy-3-methoxybenzoylacetate, m.p. 56—57 °C (from aqueous ethanol); $\nu_{max.}$(Nujol) 1 748, 1 678, and 1 600 cm^{-1}; δ($CDCl_3$; 60 MHz) 1.22 (3 H, t, *J* 7 Hz), 3.92 (2 H, s), 4.18 (2 H, q, *J* 7 Hz), 5.11 (2 H, s), 6.98 (2 H, d, *J* 9 Hz), 7.17—7.50 (5 H, m), and 7.90 (2 H, d, *J* 9 Hz); *m/z* 298 (M^+), 256, 226, 211, 107, and 91 (Found: C, 72.7; H, 6.3. $C_{18}H_{18}O_4$ requires C, 72.5; H, 6.05%).

4-Benzyloxybenzoylacetic Acid.—Ethyl 4-benzyloxybenzoylacetate was hydrolysed as described[12] for ethyl benzoylacetate (88%). The acid obtained was characterized as 4-benzyloxyacetophenone obtained on heating the β-keto acid with M-sulphuric acid (63%); m.p. 92—93 °C (from benzene–light petroleum) (lit.,[27] 93 °C); $\lambda_{max.}$ 215 and 270 nm; $\nu_{max.}$(Nujol) 1 675 and 1 600; δ($CDCl_3$) 2.55 (3 H, s), 5.14 (2 H, s), 7.04 (2 H, d, *J* 9 Hz), 7.43 (5 H, s), and 7.96 (2 H, s, *J* 9 Hz); *m/z* 226.099 57 (M^+) ($C_{15}H_{14}O_2$ requires *M*, 226.099 37).

4-Hydroxybenzoylacetic Acid (**10**).—This acid was prepared as described above for 4-hydroxy-3-methoxybenzoylacetic acid (93%) and it was characterized as 4-hydroxyacetophenone which was obtained on heating it with M-sulphuric acid (60%); m.p. 107—108 °C (lit.,[28] 109 °C); $\lambda_{max.}$(EtOH) (log ε) 280 nm (4.13); $\lambda_{max.}$(EtOH + 2M-NaOH) 238 (3.84) and 332 nm (4.33); $\nu_{max.}$(Nujol) 3 315, 1 665, and 1 603 cm^{-1}; δ($CDCl_3$) 2.61 (3 H, s), 6.98 (2 H, d, *J* 9 Hz), 7.0—7.6 (1 H, br s, exchanged with D_2O), and 7.94 (2 H, d, *J* 9 Hz); *m/z* 136.052 75 (M^+) ($C_8H_8O_2$ requires *M*, 136.052 43), 121, and 93.

4′-Benzyloxy-2-pyrrolidin-2-ylacetophenone.—This compound was prepared in 61% yield as an unstable oil from 4-benzyloxybenzoylacetic acid and 3,4-dihydro-5*H*-pyrrole as described for 4′-benzyloxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone above; $\lambda_{max.}$(EtOH) 212 and 274; $\nu_{max.}$(film) 3 380, 1 680, 1 608, and 1 582 cm^{-1}; δ($CDCl_3$; 60 MHz) 1.1—2.2 (4 H, unresolved), 2.6—3.2 (4 H, unresolved), 3.1 (1 H, s, NH), 3.3—3.8 (1 H, m), 5.1 (2 H, s), 7.41 (5 H, s), 7.0 (2 H, d, *J* 9 Hz), and 7.98 (2 H, d, *J* 9 Hz). Acetylation (*cf.* above) gave *N-acetyl-4′-benzyloxy-2-pyrrolidin-2-ylacetophenone* as a colourless oil which had the following spectral characteristics: $\nu_{max.}$(film) 1 685, 1 640, 1 610, and 1 590 cm^{-1}; δ($CDCl_3$; 60 MHz) 1.8—2.2 (4 H, unresolved), 2.05 (3 H, s), 2.3—4.0 (5 H, unresolved) 5.1 (2 H, s), 7.0 (2 H, d, *J* 10 Hz), 7.4 (5 H, s) and 8.1 (2 H, d, *J* 10 Hz); *m/z* 337.167 76 (M^+) ($C_{21}H_{23}NO_3$ requires *M*, 337.167 78), 294, 246, 226, 211, 166, 126, 91, and 83.

4′-Hydroxy-2-pyrrolidin-2-ylacetophenone (**13**).—This compound was prepared as an oil in 44% yield from 4′-benzyloxy-2-pyrrolidin-2-ylacetophenone as described for 4′-hydroxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone above; $\nu_{max.}$(film), 3 460, 1 670, and 1 610 cm^{-1}. It was characterized as its *N,O*-diacetyl derivative: $\nu_{max.}$($CHCl_3$) 1 760, 1 680, and 1 630 cm^{-1}; *m/z* 289.131 08 (M^+) ($C_{16}H_{19}NO_4$ requires *M*, 289.131 40), 246, 203, 178, and 163.

Labelled Compounds

[*A*] *Deuteriated Compounds.*—(cf. *Ref.* 13). 4-Hydroxy-3-methoxybenzoic acid (300 mg, 1.79 mmol) was heated with deuterium oxide (2.4 ml) containing triethylamine (368 mg, 3.64 mmol) at 100 °C in a sealed tube for 3 days. The reaction mixture was acidified with concentrated hydrochloric acid and extracted with ether. The ether extract was dried and the solvent was evaporated. The residue was taken up in methanol which was then evaporated; this procedure was repeated twice more. The residual [5-^{2}H]-4-hydroxy-3-methoxybenzoic acid was recrystallized from water. Mass spectral analysis showed that the material was 93% monodeuteriated; the deuterium atom was located at C-5 by proton n.m.r. (signal at δ 6.97 in $CDCl_3$-[$^2H_6$]DMSO was absent). Conversion of this material into [5-^{2}H]-4-benzyloxy-3-methoxybenzoylacetic acid by the method described above resulted in no loss of deuterium.

Similar deuteriation of 4-hydroxybenzoic acid gave [3,5-$^2H_2$]-4-hydroxybenzoic acid. Mass spectral analysis showed that the material was 76% dideuteriated and 20% monodeuteriated; the signal for the protons on C-3 and C-5 in the n.m.r.

spectrum was reduced in intensity (δ 6.95 in hexadeuterioacetone). Conversion of this material into [3,5-$^2H_2$]-4-benzyloxybenzoylacetic acid by the method described above resulted in no loss of deuterium.

[*B*] *Tritiated Compounds.*—[5-^{3}H]-4-Hydroxy-3-methoxybenzoic acid (15 mCi mmol^{-1}) and [3,5-$^3H_2$]-4-hydroxybenzoic acid (36 mCi mmol^{-1}) were prepared in a similar manner to that described for the deuteriated compounds; tritiated water (2 Ci ml^{-1}) and approx. 100 mg of each acid were used. The tritiated water was introduced to the sample plus triethylamine on a vacuum line and later removed using the same apparatus.

Preparation of [5-^{3}H]-4-hydroxy-3-methoxybenzoylacetic acid (**24**), [3,5-$^3H_2$]-4-hydroxybenzoylacetic acid (**23**), [5′-^{3}H]-4′-hydroxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone [as **22**)], and [3′,5′-$^3H_2$]-4′-hydroxy-2-pyrrolidin-2-ylacetophenone [as (**21**)] was from these acids by application of the methods described above; they were successful on a small scale. Oxalyl chloride plus a catalytic amount of dimethylformamide was used instead of thionyl chloride for the preparation of acid chlorides.

[5-^{3}H]-2-Phenacylpyrrolidine [as (**20**)] was prepared from DL-[5-^{3}H]ornithine by application of the method already described.

[*C*] ^{14}C-*Labelled Compounds.*—[5-^{14}C]-3,4-Dihydro-5H-pyrrole was prepared from DL-[5-^{14}C]ornithine. It was used to make [5-^{14}C]-2-(4′-hydroxy-3′-methoxyphenacyl)pyrrolidine [as (**22**)] and [5-^{14}C]-2-(4′-hydroxyphenacyl)pyrrolidine [as (**21**)]. The methods described above were successfully applied.

[*U-ring*-^{14}C]Benzoic acid was converted through labelled ethyl benzoylacetate into [*U-benzene ring*-^{14}C]-2-pyrrolidin-2-ylacetophenone [as (**20**)] by application of methods described above for the oxygenated analogues. [*Carbonyl*-^{14}C]benzoylacetic acid (**9**) was prepared similarly.

Precursor Feeding to T. asthmatica.—The precursors were assimilated into the plants in aqueous solution either through wicks inserted in the base of the stems of whole plants or by standing the aerial parts of plants (which were excised just above ground-level) in the aqueous solutions. The plants were greenhouse grown; feeding experiments were carried out in the summer. The amines were sufficiently soluble in water at pH 6 and solutions of this pH used in the experiments. The acids were dissolved in water. Uptake of solution was complete in 1—2 days. Water was then added to the containers periodically. The plants were allowed to grow for 10—14 days before work-up. Feeding experiments were carried out on two occasions: (i) [5-^{3}H]-Samples of (**12**) (50 μCi), (**13**) (34.9 μCi), and (**14**) (22.7 μCi) were fed to the aerial parts of the plants; incorporation of the precursors into tylophorine (**3**) was, respectively, 0.012, 0.035, and 0.023%, and into tylophorinidine (**17**), respectively, 0.1, 0.021, and 0.008%; approximately 20 mg of (**3**), 30 mg of (**16**) as its *O*-acetyl derivative, and 30 mg of (**17**) were isolated. (ii) Samples of ^{14}C and ^{3}H labelled amines, *i.e.* (**20**), (**21**), and (**22**), were used: (**20**) 15 mg, 0.08 mmol, 10.5 μCi ^{14}C, 132.3 μCi ^{3}H (ratio: 12.6); (**21**) 4.6 mg, 0.022 mmol, 12.0 μCi ^{14}C; 73.0 μCi ^{3}H, (ratio: 6.1); (**22**) 4.3 mg, 0.018 mmol, 11.4 μCi ^{14}C, 30.8 μCi ^{3}H, (ratio: 2.7); (**9**) 28 mg, 0.17 mmol, 41.3 μCi ^{14}C; (**23**) 5.4 mg, 0.03 mmol, 465 μCi ^{3}H; (**24**) 7.8 mg, 0.04 mmol, 36.7 μCi ^{3}H; in this set of experiments inactive tyrosine (*ca.* 20 mg) was assimilated along with each of the labelled precursors; 22—37 mg of tylophorine (**3**), 17—40 mg of the acetylated derivative of tylophorinine (**16**), and 15—30 mg of tylophorinidine (**17**) were isolated [except in the case of (**22**) when no (**17**) was isolated]; the results are given in the Table.

Isolation and Purification of Alkaloids.—Typically, plant material (*ca.* 1 kg) was macerated with ethanol containing 1% acetic acid (*ca.* 10 l). The mixture was allowed to stand for 3 days and was then filtered. Evaporation of the solvent left a thick black residue which was taken up in 0.5M-sulphuric acid (*ca.* 600 ml). The acidic extracts were concentrated under reduced pressure to *ca.* 200 ml. The solution was washed with ether (3 × 200 ml) and then cooled and basified with concentrated aqueous ammonia. This solution was extracted with chloroform (6 × 100 ml). The combined extracts were dried and the solvent was evaporated to leave a brown oil (*ca.* 1 g).

The crude mixture of alkaloids was chromatographed. Tylophorine and tylophorinine co-eluted with 2% methanol in chloroform; tylophorinidine (*ca.* 100 mg) was eluted with 10% methanol in chloroform. Difficulties in crystallizing the tylophorinidine could be overcome by converting it into *O*-methyltylophorinidine with ethereal diazomethane. The *O*-methyltylophorinidine thus prepared was purified by preparative t.l.c. (20% MeOH in $CHCl_3$) and was recrystallized from ethanol–chloroform.

The mixture of tylophorine and tylophorinine (*ca.* 300 mg) was treated at 100 °C with acetic anhydride (5 ml) and a few drops of pyridine for 2 h. After evaporation the residue was chromatographed with chloroform to give acetyltylophorinine; tylophorine was eluted with 5% methanol in chloroform. Tylophorine was recrystallized from ethanol–chloroform. The acetyltylophorinine was further purified by preparative t.l.c. (10% MeOH in $CHCl_3$) and was then recrystallized from ethyl acetate.

The alkaloids were crystallized to constant radioactivity or until the level of radioactivity was negligible.

Degradation of the 2-*Pyrrolidin*-2-*ylacetophenone* (**28**) *formed from* [5-^{14}C]*Ornithine* (**26**).—Spectral and analytical data which are given below were obtained on unlabelled material.

(*a*) 1-*Benzoyl*-5-*dimethylaminopent*-1-*ene* (**30**). [5-^{14}C]-2-Pyrrolidin-2-ylacetophenone (**28**) (90 mg, 29.7 μCi) was obtained from DL-[5-^{14}C]ornithine (**26**) and benzoylacetic acid in 24% yield; *N*-methylation gave (**29**). The methods used were those described earlier.[12] The radioactive (**29**) obtained was diluted with inactive *N*-methyl-2-phenacylpyrrolidine (3.5 g) prepared by another route.[17] A solution of this diluted material in ether was treated overnight at room temperature with an excess of methyl iodide. The methiodide of (**29**) precipitated as a white hygroscopic solid. The supernatant liquid was decanted and the last traces of liquid were removed under reduced pressure. This material was characterized as the picrate, m.p. 136—138 °C (from propan-2-ol) (Found: C, 54.05; H, 5.15; N, 12.6. $C_{20}H_{22}N_4O_8$ requires C, 53.81; H, 4.93; N, 12.56%). The radioactive material was crystallized to constant activity (0.80 μCi mmol^{-1}).

A solution of the methiodide (2.6 g; quantities here and below are for unlabelled material) in water (50 ml) was basified by the dropwise addition of 2M-aqueous sodium hydroxide; the reaction mixture was kept cool. The mixture was extracted with chloroform; the extract was dried and evaporated to leave a yellow oil which was chromatographed on neutral alumina (100 g) with benzene–chloroform (1:1) as eluant. 1-*Benzoyl*-5-*dimethylaminopent*-1*ene* (**30**) was obtained as a pale yellow oil (41%); $\nu_{max.}$(film) 1 668, 1 648, 1 618, and 1 595 cm^{-1}; δ($CDCl_3$) 1.55—1.90 (2 H, m), 2.05—2.65 (4 H, unresolved), 2.23 (6 H, s), 6.75—7.15 (2 H, m), 7.33—7.65 (3 H, m), and 7.85—8.05 (2 H, m); *m/z* 217.147 10 (M^+) ($C_{14}H_{19}NO$ requires *M*, 217.146 66), 146, 121, 112, 105, 96, 77, 72, 71, and 58.

(*b*) 1-*Benzoyl*-5-*dimethylaminopentane* (**31**). The pentene (**30**) (0.9 g) in dry ethanol (50 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladium on charcoal (100 mg) until hydrogen uptake ceased (3

h). The mixture was filtered (Celite) and the solvent was evaporated from the filtrate to give *1-benzoyl-5-dimethylaminopentane* (**31**) as a pale yellow oil (95% yield); $\nu_{max.}$(film) 1 680 and 1 595 cm^{-1}; δ($CDCl_3$) 1.25—2.0 (6 H, unresolved) 2.2—2.8 (2 H, m), 2.25 (6 H, s), 3.00 (2 H, t, *J* 7 Hz), 7.4—7.7 (3 H, m), and 7.98 (2 H, m); *m/z* 219.161 67 (M^+) ($C_{14}H_{21}NO$ requires *M*, 219.162 31), 120, 114, 105, 100, 77, and 58.

(*c*) *1-Phenyl-6-dimethylaminohexan-1-ol* (**32**). To a solution of (**31**) (0.8 g) in dry methanol (25 ml) was added, in portions, sodium borohydride (0.25 g). The mixture was stirred overnight. The methanol was evaporated and the residue was taken up in dilute sulphuric acid. The solution was washed with ether, then it was cooled and basified with concentrated aqueous ammonia. Extraction with ether gave *1-phenyl-6-dimethylaminohexan-1-ol* (**32**) as a colourless oil (88% yield); $\nu_{max.}$(film) 3 360 and 1 600 cm^{-1}; δ($CDCl_3$) 0.8—2.8 (10 H, unresolved), 2.09 (6 H, s), 4.56 (1 H, t, *J* 7 Hz), 4.82 (1 H, br s, exchanged with D_2O), and 7.29 (5 H, s); *m/z* 221.178 09 (M^+) ($C_{14}H_{23}NO$ requires *M*, 221.177 96).

(*d*) *1-Phenylhex-6-en-1-ol* (**34**). To a solution of (**32**) (600 mg) in methanol (5 ml) was added hydrogen peroxide (2.5 ml, 100 vol.); the mixture was allowed to stand overnight. Excess of hydrogen peroxide was destroyed with 10% palladium on charcoal. The mixture was filtered (Celite). Evaporation of the filtrate gave the *1-phenyl-6-dimethylaminohexan-1-ol* N-*oxide* (**33**); $\nu_{max.}$(film) 3 300 and 1 600 cm^{-1}; δ(CD_3OD) 1.15—2.2 (8 H, unresolved), 2.9—3.5 (2 H, br m), 3.09 (6 H, s), 4.63 (1 H, t, *J* 7 Hz), and 7.33 (5 H, s); *m/z* no M^+, 221.177 65 ($C_{14}H_{23}NO$ which is M^+-oxygen, requires *m/z* 221.177 96), 120, 107, 77, and 58. The *N*-oxide (**33**)was heated in an oil-bath (170 °C) *in vacuo* (below 10^{-4} mmHg) and the distillate was collected on a cold finger. This distillate was taken up in chloroform. This solution was washed with M-sulphuric acid and was then dried. Evaporation gave *1-phenylhex-6-en-1-ol* (**34**) as a colourless oil (77% yield); $\nu_{max.}$(film) 3 360, 1 640, and 1 600 cm^{-1}; δ($CDCl_3$) 1.1—2.0 (4 H, m), 2.06 (2 H, q, *J* 7 Hz), 2.33 (1 H, br s, exchanged with D_2O), 4.61 (1 H, t, *J* 7 Hz), 4.85—5.2 (2 H, m), 5.55—6.05 (1 H, m), and 7.30 (5 H, s); *m/z* 176.120 81 (M^+) ($C_{12}H_{16}O$ requires *M*, 176.121 09), 158, 133, 120, 117, 107, 80, and 77.

(*e*) *Degradation of* 1-*phenylhex-6-en-1-ol* (**34**) *to give formaldehyde.* To a solution of (**34**) (410 mg) in ether (20 ml) was added osmium tetraoxide (*ca.* 10 mg). The mixture was stirred until it turned deep brown. Water (20 ml) was added, followed, in several portions, by sodium metaperiodate (1.0 g). The mixture was stirred in the dark for 3 h. More water was added (*ca.* 20 ml) and the mixture was distilled. To the distillate (*ca.* 40 ml) was added dimedone (700 mg); the solution obtained was warmed for a few minutes. The dimedone derivative of formaldehyde crystallized on standing. It was collected and recrystallized (aqueous methanol), m.p. 193—194 °C. The radioactive material was crystallized to constant activity: 0.79 μCi $mmol^{-1}$.

Acknowledgements

We thank Dr. T. R. Govindachari for *T. asthmatica* plants, the S.E.R.C. for a studentship (F. B. J.) and financial support, and the University of Leeds for a demonstratorship (I. T. N.). We thank Mr. P. C. Wormald for full physical data on 4′-hydroxy-3′-methoxy-2-pyrrolidin-2-ylacetophenone (**14**) [(±)-norruspolinone] and its *N,O*-diacetyl derivative, and Dr. M. Hesse for comparing our spectra with those he obtained for natural norruspolinone and its *N,O*-diacetyl derivative.

References

1 J. H. Russel, *Naturwissenschaften*, 1963, **50**, 443; K. V. Rao, *J. Pharm. Sci.*, 1970, **59**, 1608; T. R. Govindachari, N. Viswanathan, J. Radhakrishnan, B. R. Pai, S. Natarajan, and P. S. Subramaniam, *Tetrahedron*, 1973, **29**, 891.
2 N. K. Hart, S. R. Johns, and J. A. Lamberton, *Aust. J. Chem.*, 1968, **21**, 2579.
3 N. R. Farnsworth, N. K. Hart, S. R. Johns, J. A. Lamberton, and W. Messmer, *Aust. J. Chem.*, 1969, **22**, 1805; N. K. Hart, S. R. Johns, and J. A. Lamberton, *ibid.*, 1968, **21**, 1397.
4 F. Roessler, D. Ganzinger, S. Johne, E. Schöpp, and M. Hesse, *Helv. Chim. Acta*, 1978, **61**, 1200.
5 R. B. Herbert and F. B. Jackson, *J. Chem. Soc., Chem. Commun.*, 1977, 955.
6 D. S. Bhakuni and V. K. Mangla, *Tetrahedron*, 1981, **37**, 401.
7 N. B. Mulchandani, S. S. Iyer, and L. P. Badheka, *Phytochemistry*, 1971, **10**, 1047.
8 N. B. Mulchandani, S. S. Iyer, and L. P. Badheka, *Phytochemistry*, 1976, **15**, 1697.
9 N. B. Mulchandani, S. S. Iyer, and L. P. Badheka, *Phytochemistry*, 1969, **8**, 1931.
10 R. B. Herbert, unpublished results.
11 R. B. Herbert, in 'Rodds Chemistry of Carbon Compounds,' 2nd edn., ed. S. Coffey, Elsevier, Amsterdam, 1980, vol. IV Part L, p. 291; R. B. Herbert, 'The Biosynthesis of Secondary Metabolites,' Chapman and Hall, London, 1981.
12 J. E. Cragg, R. B. Herbert, F. B. Jackson, C. J. Moody, and I. T. Nicolson, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2477.
13 G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
14 R. B. Herbert and P. C. Wormald, *J. Chem. Research* (*S*), 1982, 299; (*M*), 3001.
15 W. B. Jakoby and J. Fredericks, *J. Biol. Chem.*, 1959, **234**, 2145.
16 R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897.
17 F. Galinovsky, A. Wagner, and R. Weiser, *Monatsh. Chem.*, 1951, **82**, 551.
18 R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, 1969, **47**, 445.
19 R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, 1967, **45**, 1275.
20 B. J. Hunt and W. Rigby, *Chem. Ind.* (*London*), 1967, 1868.
21 H. Pacheco and A. Grouiller, *Bull. Soc. Chim. Fr.*, 1965, 779.
22 K. Kratzl and E. Kvasnicka, *Monatsh. Chem.*, 1952, **83**, 18.
23 K. Kratzl and G. E. Miksche, *Monatsh. Chem.*, 1963, **94**, 434.
24 A. H. Sommers and A. W. Weston, *J. Am. Chem. Soc.*, 1951, **73**, 5749.
25 F. Tiemann, *Ber.*, 1891, **24**, 2855.
26 E. L. Eliel and R. P. Anderson, *J. Am. Chem. Soc.*, 1952, **74**, 547.
27 H. M. Priestley and E. Moness, *J. Org. Chem.*, 1940, **5**, 355.
28 K. Nakazawa and S. Matsuura, *J. Pharm. Sci.*, 1954, **74**, 69.

Received 22*nd August* 1983; *Paper* 3/1473