The Biosynthesis of *Lobelia* Alkaloids. Part III.[1] Intermediates in the Biosynthesis of Lobeline; Biosynthesis of 8,10-Diethyl-lobelidione

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Cinnamic acid and 3-hydroxy-3-phenylpropanoic acid are incorporated into lobeline {2-[6-(β-hydroxyphenethyl)-1-methyl-2-piperidyl]acetophenone} in *Lobelia inflata*. Lysine, pentane-1,5-diamine (cadaverine), and 3,4,5,6-tetrahydropyridine are also incorporated into lobeline; their relative incorporations show that pentane-1,5-diamine is not an intermediate in the biosynthesis of this alkaloid from lysine. 8,10-Diethyl-lobelidione [1,1'-(1-methylpiperidine-2,6-diyl)di(butan-2-one)] is shown, by tracer experiments, to be derived from lysine and acetate in *L. inflata* plants.

THE *Lobelia* alkaloids contain a piperidine or a 1,2,3,6-tetrahydropyridine nucleus [2] and may be divided into three groups according to the pattern of substitution at C-2 and C-6: (*a*) those with C_6C_2 units at C-2 and C-6 of the piperidine nucleus, such as lobeline (VII); (*b*) those with a C_4 unit at C-2 and a C_6C_2 unit at C-6 of the piperidine or 1,2,3,6-tetrahydropyridine nucleus such as lelobeline or lobinine; and (*c*) those with C_4 units at both C-2 and C-6 such as 8,10-diethyl-lobelidione (XVI).

These alkaloids can be considered to arise from lysine and phenylalanine or acetate,[3] or from benzoic acid and acetate, or from acetate alone.[4] Tracer studies have shown that lobeline is derived from phenylalanine and lysine.[5] A possible biosynthetic pathway is outlined in Scheme 1. Phenylalanine (I) on deamination yields *trans*-cinnamic acid (II). The necessary enzyme, phenylalanine ammonia-lyase has been isolated from plant sources.[6] Hydroxylation of cinnamic acid gives 3-hydroxy-3-phenylpropanoic acid (III), which has been isolated from *L. inflata*.[7] Oxidation yields benzoylacetic acid (IV), the presumed intermediate in the biosynthesis of C_6C_1 compounds from cinnamic acid and its phenolic derivatives.[8] Condensation of (IV) with 2,3,4,5-tetrahydropyridine (X) gives the aminoketone (V), which on oxidation and reaction with another molecule of benzoylacetic acid gives lobelanine (VI). Reduction of one of the carbonyl groups of lobelanine yields lobeline (VII). We have previously demonstrated the incorporation of labelled lobelanine into lobeline in high yield.[1]

To delineate further the pathway from phenylalanine to lobeline the following feeding experiments were undertaken. In separate experiments (±)-[3-^{14}C]phenylalanine (total activity 0·1 mCi) and [3-^{14}C]cinnamic acid (total activity 0·1 mCi) were fed, by the wick method, to twelve *L. inflata* plants. The plants, which were approaching the flowering stage, were grown on for 10 days and then harvested. After the addition of inactive lobeline as carrier (400 mg), active lobeline was isolated as described previously [5] and purified to constant specific activity. In a third experiment 3-hydroxy-3-phenyl[3-^{14}C]propanoic acid (total activity 0·1

[1] D. G. O'Donovan and T. Forde, *J. Chem. Soc.* (*C*), 1971, 2889.

[2] H. Wieland, *Ber.*, 1921, **54**, 1784; H. Wieland, C. Schopf, and W. Hermsen, *Annalen*, 1925, **444**, 50; H. Wieland, W. Koshara, E. Dane, J. Renz, W. Schwarze, and W. Linde, *ibid.*, 1939, **540**, 103; C. Schopf and T. Kaufmann, *ibid.*, 1957, **608**, 88.

[3] R. Robinson, *J. Chem. Soc.*, 1917, **3**, 876.

[4] E. Leete, in 'Biogenesis of Natural Products,' ed. P. Bernfeld, Pergamon, Oxford, 1963, p. 752.

[5] M. F. Keogh and D. G. O'Donovan, *J. Chem. Soc.* (*C*), 1970, 2470.

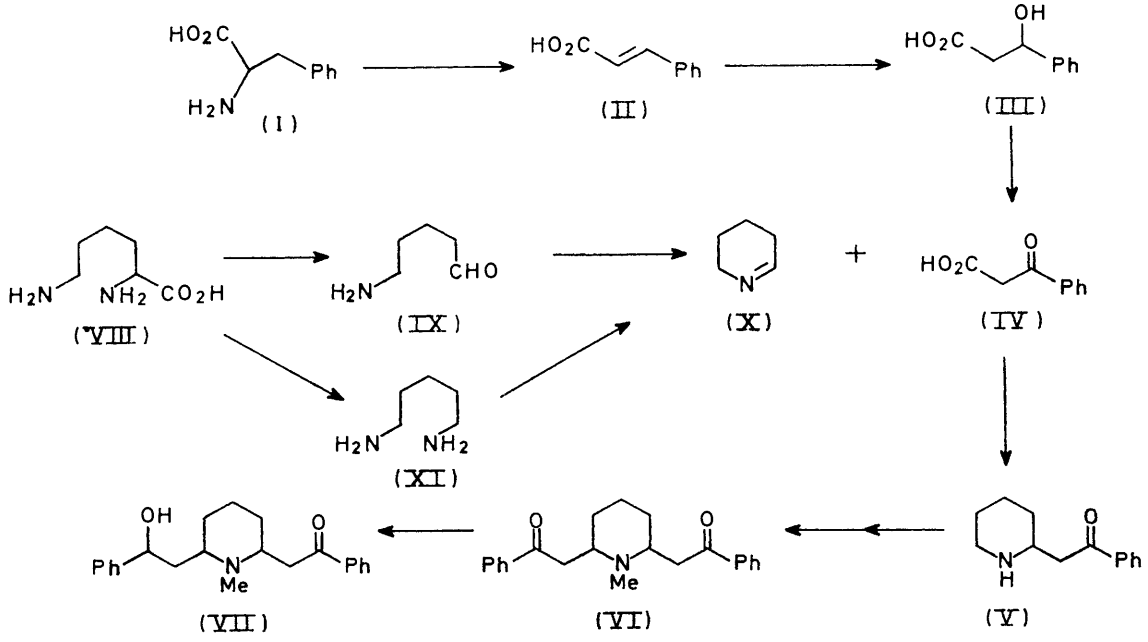
[6] J. Koughal and E. E. Conn, *J. Biol. Chem.*, 1961, **236**, 2672.

[7] H. Wieland, W. Koschara, E. Dane, J. Renz, W. Schwarze, and W. Linde, *Annalen*, 1939, **540**, 103.

[8] M. H. Jenk and G. Mueller, *Z. Naturforsch.*, 1964, **196**, 398; G. G. Gross and M. H. Jenk, *ibid.*, 1966, **216**, 683; H. Grisebach and K. O. Vollmer, *ibid.*, 1963, **186**, 753; S. Z. El-Basyouni, D. Chen, R. K. Ibrahim, A. C. Neisch, and G. N. Towers, *Phytochemistry*, 1964, **3**, 485; D. J. Bennett and G. W. Kirby, *J. Chem. Soc.* (*C*), 1968, 442.

μCi) was similarly fed to twelve *L. inflata* plants, and after addition of inactive lobeline as carrier (300 mg) active lobeline was again isolated. Percentage incorporations are reported in Table 1.

The active lobeline from the cinnamic acid and 3-hydroxy-3-phenylpropanoic acid feeds was oxidised



SCHEME 1

to benzoic acid with alkaline permanganate. The concordance between the specific activities of lobeline and the isolated benzoic acid (Table 2) in each case, showed that these precursors are specifically incorporated into lobeline. The incorporation figures for phenylalanine, cinnamic acid, and 3-hydroxy-3-phenylpropanoic acid (0·05, 0·11, and 3·8%, respectively) validate the pathway from phenylalanine to lobeline shown in Scheme 1.

TABLE 1

Incorporation of tracers into lobeline and 8,10-diethyllobelidione

Tracer	Amount fed (mCi)	Incorporation (%) Lobeline	8,10-Diethyllobelidione
(±)-[3-^{14}C]Phenylalanine	0·1	0·05	
[3-^{14}C]Cinnamic acid	0·1	0·11	
3-Hydroxy-3-phenyl[3-^{14}C]-propionic acid	0·0001	3·80	
[2-^{14}C]Lysine	0·1	0·05	
[1,5-$^{14}C_2$]Pentane-1,5-diamine	0·05	0·005	
2,3,4,5-Tetrahydro[2-^{14}C]-pyridine	0·07	3·30	
[2-^{14}C]Lysine	0·2		0·05
Sodium[1-^{14}C]acetate	0·2		0·009

It has been demonstrated that lysine (VIII) is incorporated unsymmetrically into a number of alkaloids, *e.g.* sedamine,[9] anabasine,[10] and *N*-methylisopelletierine,[11] *via* 5-aminopentanal (IX)[12] and 2,3,4,5-tetrahydropyridine (X).[13] Although pentane-1,5-diamine (cadaverine) (XI) has also been incorporated into these alkaloids,[14] their biosynthesis from this precursor represents an aberrant pathway. Recently it has been reported that lysine is incorporated symmetrically into decodine.[15]

The symmetrical incorporation of lysine into lobeline, previously reported,[5] can be explained by the intervention in the biosynthetic pathway of the symmetrical intermediate lobelanine (VI), which has been shown to be incorporated into lobeline in high yield.[1] This result however does not preclude a precursor role for pentane-1,5-diamine in the biosynthesis of this alkaloid. To investigate the role of this diamine in the biosynthesis of lobeline, the following feeding experiments were undertaken.

TABLE 2

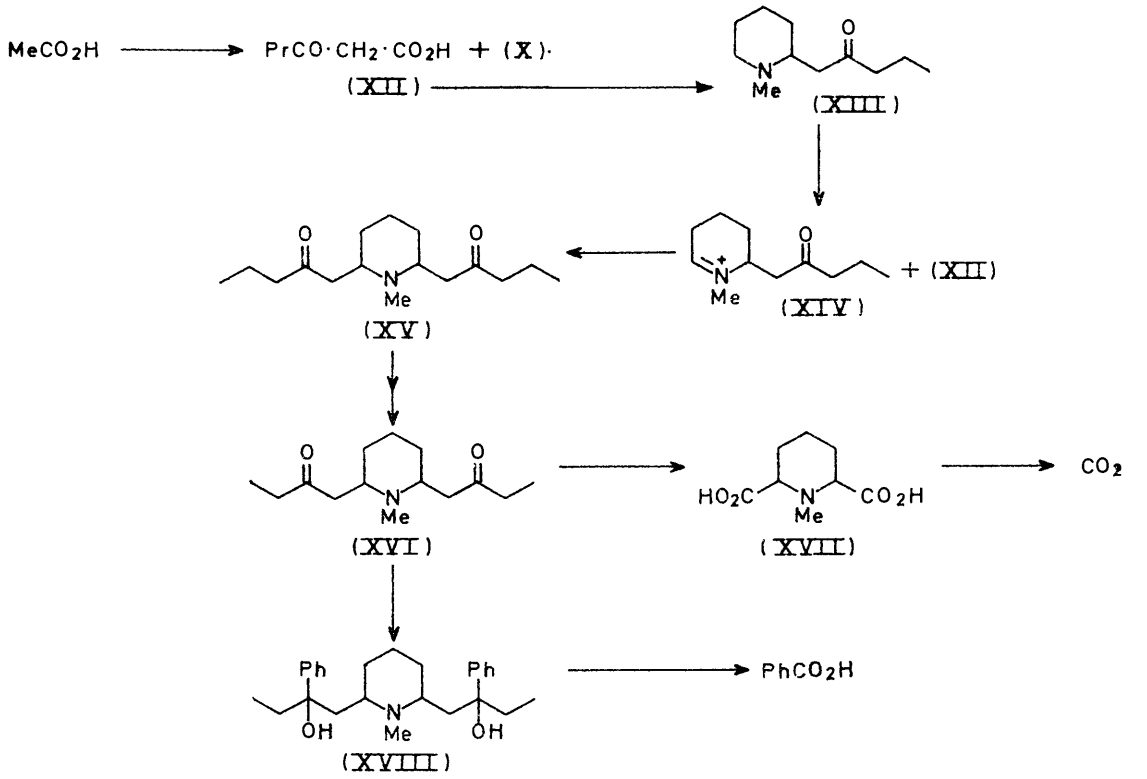
Activities of lobeline and its degradation products (disint. min^{-1} mmol^{-1})

Compound	(a) Cinnamic acid feed	(b) 3-Hydroxy-3-phenylpropanoic acid feed
Lobeline	7·28 × 10^4	8·69 × 10^3
Benzoic acid	3·53 × 10^4	4·80 × 10^3

(±)-[2-^{14}C]Lysine (total activity 0·1 mCi), [1,5-$^{14}C_2$]-pentane-1,5-diamine (total activity 0·05 mCi), and

[9] R. N. Gupta and I. D. Spenser, *J. Biol. Chem.*, 1969, **244**, 88.
[10] E. Leete, *J. Amer. Chem. Soc.*, 1956, **78**, 3520.
[11] M. F. Keogh and D. G. O'Donovan, *J. Chem. Soc.* (*C*), 1970, 1792.
[12] E. Leete and M. R. Chedekal, *Phytochemistry*, 1972, **11**, 2751.
[13] E. Leete, *J. Amer. Chem. Soc.*, 1969, **91**, 1697.
[14] K. Mothes and H. Schütte, *Angew. Chem. Internat. Edn.*, 1963, **2**, 341.
[15] S. H. Koo, R. N. Gupta, I. D. Spenser, and J. T. Wrobel, *Chem. Comm.*, 1970, 396.

2,3,4,5-tetrahydro[2-^{14}C]pyridine [16] (total activity 0·07 mCi) were fed, in separate experiments, to twelve *L. inflata* plants in the same stage of growth. The plants were grown on for 14 days and, after the addition of inactive lobeline (300 mg) as carrier, active lobeline was isolated and purified to constant specific activity. Incorporations from the lysine, pentanediamine, and tetrahydropyridine feeds were 0·05, 0·005, and 3·3%, respectively. These results, together with those reported previously,[1] show that although pentanediamine is incorporated into lobeline it is not a normal intermediate between lysine and the alkaloid. Lobeline



SCHEME 2

wick method. The tracer solution was absorbed within 48 h. The plants were watered through the wick for 48 h and then grown on normally for a further 10 days. The plants were harvested, and inactive 8,10-diethyl-lobelidione (95 mg) was added as carrier. Active 8,10-diethyl-lobelidione was isolated as the reineckate and purified to constant specific activity (incorporation 0·05%). In a second experiment sodium [1-^{14}C]acetate (total activity 0·2 mCi) was administered to twelve *L. inflata* plants and, after the addition of inactive 8,10-diethyl-lobelidione (100 mg) as carrier, active alkaloid was again isolated as its reineckate (incorporation 0·009%).

then falls into the major class of piperidine alkaloids whose biosynthesis from lysine proceeds unsymmetrically *via* 2,3,4,5-tetrahydropyridine.

In agreement with the foregoing results, *Lobelia* alkaloids of the third group can be considered to arise by the pathway shown in Scheme 2. Lysine (VIII) is converted *via* 5-aminopentanal (IX) into 2,3,4,5-tetrahydropyridine (X) as in Scheme 1. Condensation of (X) with 3-oxohexanoic acid (XII), derived from acetate, yields the amino-ketone (XIII); oxidation of (XIII) to the 2,3,4,5-tetrahydropyridine derivative (XIV) and condensation with another molecule of (XII) yields the dione (XV). Loss of the terminal methyl groups of the side chains of (XV) yields 8,10-diethyl-lobelidione (XVI).

To validate this scheme for 8,10-diethyl-lobelidione, (±)-[2-^{14}C]lysine (total activity 0·2 mCi) was administered to twelve *L. inflata* plants, prior to flower, by the

The 8,10-diethyl-lobelidione from the lysine feed, regenerated from the reineckate, was oxidised to (1-methylpiperidine-2,6-dicarboxylic acid) (XVII) with chromium trioxide–sulphuric acid. The acid was subjected to a Schmidt reaction yielding carbon dioxide, collected as barium carbonate. The concordance between the specific activities of 8,10-diethyl-lobelidione and its degradation products (Table 3) shows that the piperidine ring is derived from lysine.

The 8,10-diethyl-lobelidione from the acetate feed was treated with phenyl-lithium and the resultant carbinol (XVIII) was oxidised directly, with permanganate, to benzoic acid. The specific activity of the benzoic acid (Table 3) was one quarter that of the 8,10-diethyl-lobelidione showing that both side chains of the alkaloid were derived from acetate.

[16] R. N. Gupta and I. D. Spenser, *Canad. J. Chem.*, 1969, **445**, 47.

TABLE 3

Specific activities of 8,10-diethyl-lobelidione and its degradation products (disint. min^{-1} $mmol^{-1}$ × 10^{-5})

(a) Lysine feed		(b) Acetate feed	
8,10-Diethyl-lobelidione reineckate	5·27	8,10-Diethyl-lobelidione reineckate	9·02
1-Methylpiperidine-2,6-dicarboxylic acid	5·11	Benzoic acid	2·21
Barium carbonate	0		

EXPERIMENTAL

M.p.s are corrected. Radioactivity assays were carried out with a Nuclear Chicago Unilux II liquid scintillation counter by use of the usual scintillants; the results were processed by an off-line Olivetti Programma 101 computer, corrections being made for background and quenching.

Administration of Tracers to L. inflata *and Isolation of Lobeline.*—(±)-[3-^{14}C]Phenylalanine (total activity 0·1 mCi) was administered, by the wick method, to twelve *L. inflata* plants. The plants were grown on for 10 days and then harvested. Active lobeline was isolated as described previously.[5] In further separate experiments [3-^{14}C]-cinnamic acid (total activity 0·1 mCi) and 3-hydroxyphenyl[3-^{14}C]propanoic acid (total activity 0·1 μCi) were fed to twelve *L. inflata* plants, which were grown on for 10 days, and active lobeline was again isolated. In a second series of experiments (±)-[2-^{14}C]lysine (total activity 0·1 mCi), [1,5-^{14}C]pentane-1,5-diamine (total activity 0·05 mCi), and 2,3,4,5-tetrahydro[2-^{14}C]pyridine (total activity 0·07 mCi) were fed to twelve *L. inflata* plants, in a comparable state of growth, in separate experiments. The plants were grown on for 10 days and then harvested. Active lobeline was isolated in each case.

Administration of Tracers to L. inflata *and Isolation of 8,10-Diethyl-lobelidione.*—(±)-[6-^{14}C]Lysine (total activity 0·2 mCi) in aqueous solution (12 ml) was fed to twelve *L. inflata* plants, prior to flowering, by the wick method. The tracer solution was absorbed within 48 h. The plants were watered through the wick for 48 h and grown on for a further 10 days. They were then harvested and homogenised in a Waring blendor with ethanol (500 ml), and after the addition of inactive 8,10-diethyl-lobelidione (95 mg) as carrier the mixture was kept at 4° for 3 days. The mixture was filtered through cloth and the filtrate concentrated to *ca.* 10 ml. Dilute hydrochloric acid (20 ml) was added and the solution extracted with ether. The aqueous solution was basified with ammonia and extracted with chloroform (200 ml). Evaporation of the dried (Na_2SO_4) chloroform solution yielded an orange oil (147 mg). The oil was taken up in hydrochloric acid (20%) and saturated aqueous ammonium reineckate was added dropwise. 8,10-Diethyl-lobelidione reineckate (210 mg) separated overnight; m.p. 192—195° (decomp.) (from aqueous ethanol).

The reineckate, dissolved in water–acetone (1 : 1; 40 ml), was filtered slowly through a column of Amberlite IRA-400 (Cl^-) resin (28 × 1·2 cm). 8,10-Diethyl-lobelidione hydrochloride was washed through in the solvent. The eluate was evaporated until an aqueous solution remained. Basification, extraction with chloroform, and evaporation of the dried (Na_2SO_4) extract yielded pure 8,10-diethyl-lobelidione (97 mg), identical (t.l.c. and i.r. spectrum) with an authentic specimen.[17]

[17] C. Schopf and G. Lehmann, *Annalen*, 1935, **518**, 1.

In a second experiment sodium [1-^{14}C]acetate (total activity 0·2 mCi) was similarly administered to nine *L. inflata* plants and, after the addition of 8,10-diethyl-lobelidione (100 mg) as carrier, pure active 8,10-diethyl-lobelidione (77 mg) was isolated as above.

Degradation of Lobeline from Cinnamic Acid and 3-Hydroxy-3-phenylpropanoic Acid Feeds.—Lobeline (40 mg) was refluxed for 10 h with a solution of potassium permanganate (100 mg) and potassium hydroxide (50 mg) in water (50 ml). The mixture was allowed to cool and ethanol (5 ml) was added. The mixture was filtered to remove inorganic residues and the basic filtrate was extracted with ether. The solution was acidified with concentrated hydrochloric acid and again extracted with ether (200 ml). The latter dried (Na_2SO_4) ethereal solution was evaporated to yield impure benzoic acid, which was purified by sublimation and recrystallisation from water; yield 10 mg; m.p. and mixed m.p. 122°.

Degradation of 8,10-Diethyl-lobelidione.—*1-Methylpiperidine-2,6-dicarboxylic acid* (XVII). 8,10-Diethyl-lobelidione (90 mg) was dissolved in sulphuric acid (50%; 30 ml) and chromium trioxide (350 mg) in water (30 ml) was added. The mixture was heated at 90° for 4 h and cooled to room temperature; water (10 ml) was then added and the solution extracted with ether (extract discarded). Sulphur dioxide was passed through the solution, which was then heated to remove the excess of gas. Sulphate ion was removed by dropwise addition of barium hydroxide solution. The mixture was filtered and the filtrate made alkaline with ammonium hydroxide and evaporated to a small volume. The solution was applied to a column of Amberlite IR 120 (H^+) resin (2 × 30 cm). The column was washed with water and eluted with 3% ammonium hydroxide. Evaporation of the eluate gave 1-methylpiperidine-2,6-dicarboxylic acid (18 mg), m.p. 227—230° (decomp.).

Schmidt reaction on the acid (XVII). The acid (XVII) (30 mg) was cooled in ice and 100% sulphuric acid (0·5 ml) was added. When the solid had dissolved, sodium azide (40 mg) was added and the flask was connected to a gas train containing a sulphur dioxide trap. The system was flushed with pure nitrogen and a carbon dioxide trap was added to the system. The flask was heated to 80° for 1 h. Carbon dioxide was flushed from the system with pure nitrogen. The collected barium carbonate (8 mg) was washed with ethanol and ether.

Formation of the carbinol (XVIII) *and oxidation to benzoic acid.* 8,10-Diethyl-lobelidione (70 mg) in dry tetrahydrofuran (10 ml) was added to an excess of phenyl-lithium. The solution was stirred, under nitrogen, at room temperature for 24 h. The lithium complex was hydrolysed with an excess of M-hydrochloric acid. The aqueous layer was separated, washed with ether, and basified, and the basic solution was extracted with ether (250 ml). The ethereal extract was dried (Na_2SO_4) and evaporated to yield the carbinol as a brown oil.

Without further purification the oil (54 mg) was refluxed with aqueous 10% potassium permanganate (100 ml) for 6 h. The solution was cooled and ethanol (3 ml) was added to decompose the 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 (10 mg), which was purified by recrystallisation from water and by sublimation; m.p. 120—121°.

3-*Hydroxy*-3-*phenyl*[3-^{14}C]*propanoic Acid*.—To [*carbonyl*-^{14}C]benzaldehyde (85 mg) in benzene (20 ml) were added zinc dust (55 mg) and menthyl bromoacetate (200 mg).[18] The mixture was refluxed for 2 h, then poured on ice and acidified with 5N-sulphuric acid. The solution was extracted with ether (200 ml) and the dried (Na_2SO_4) organic mixture was evaporated to yield an oil, which was refluxed with 2·5N-potassium hydroxide (5 ml) for 2 h. The cooled solution was extracted with ether, then acidified with sulphuric acid, and again extracted with ether (100 ml). The dried ethereal extract was evaporated to yield 3-hydroxy-3-phenylpropanoic acid (60 mg), which was recrystallised to constant specific activity from aqueous ethanol; m.p. 89—90° (Found: C, 65·3; H, 6·3. Calc. for $C_9H_{10}O_3$: C, 65·1; H, 6·0%).

We thank the Director, Kew Royal Botannical Gardens for a supply of *L. inflata* seeds. This work was carried out during the tenure (by D. J. L., E. F., and P. G.) of State Maintenance Allowances for research.

[4/832 *Received*, 24*th April*, 1974]

[18] R. Smiles, *J. Chem. Soc.*, 1905, **87**, 450.