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BIOSYNTHESIS OF THE 3-ETHYLCHROMONE PHYTOALEXIN LATHODORATIN IN *LATHYRUS ODORATUS*

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Key Word Index—*Lathyrus odoratus*; Leguminosae; biosynthesis; phytoalexin; lathodoratin; chromone.

Abstract—Feeding experiments with ^{14}C- and ^{13}C-labelled precursors have demonstrated that the phloroglucinol ring of the 3-ethylchromone phytoalexin lathodoratin from cupric sulphate-induced pods of sweet pea (*Lathyrus odoratus*) has a polyketide origin. The remaining five carbon 'isoprene unit' is derived from the amino acid isoleucine which provides the starter unit, probably 2-methylbutanoate, for acetate–malonate chain extension. The pterocarpan phytoalexin pisatin is produced along with lathodoratin, and precursors were incorporated in agreement with earlier studies. Two minor 3-ethylchromones were also synthesized on cupric sulphate treatment of the pods. These were the previously reported 7-*O*-methyl ether of lathodoratin (methyl-lathodoratin) and a new compound, the 5,7-di-*O*-methyl ether (dimethyl-lathodoratin). Dimethyl-lathodoratin is the most fungitoxic of the 3-ethylchromone phytoalexins.

INTRODUCTION

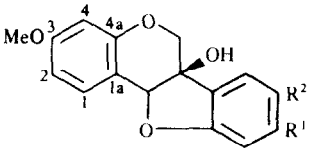
Many plants synthesize phytoalexins as a result of fungal infection or other forms of stress [1]. Plants in the Leguminosae usually synthesize phytoalexins that are isoflavonoid in nature, e.g. isoflavanones, isoflavans and pterocarpans [2], but a number of species are known to accumulate non-isoflavonoid materials instead of, or in addition to isoflavonoids. Species of *Vicia* and *Lens* produce furanoacetylenes such as wyerone [2], and stilbene derivatives are synthesized by species of *Arachis* [2]. A systematic study of phytoalexin production in the genus *Lathyrus* has shown that almost all of the species examined produce isoflavonoid phytoalexins in response to fungal infection [3]. The pterocarpan pisatin (**1**) was the major phytoalexin produced in most species, but other pterocarpans, e.g. medicarpin, maackiain, variabilin (**2**), nissolin and methylnissolin were observed in some cases. Two species examined were atypical, however, *Lathyrus odoratus* (sweet pea) and *L. hirsutus* accumulated the 3-ethylchromone phytoalexins lathodoratin (**3**) and methyl-lathodoratin (**4**) [4], in addition to pisatin and traces of other pterocarpans. In a further study [5], two α-hydroxydihydrochalcone stress metabolites, odoratol (**6**) and methylodoratol (**7**) were also identified in extracts of *L. odoratus* tissues.

The 3-ethylchromone structures of lathodoratin and methyl-lathodoratin are rather unusual and do not relate readily to other classes of natural chromones. Most simple chromones encountered in nature have a substituent at C-2 rather than C-3, and are good examples of polyketide structures derived from acetate–malonate. Thus, the 2-methylchromone eugenin (**8**) is a phytoalexin from carrot (*Daucus carota*; Umbelliferae) and is formed from acetate in accordance with biosynthetic predictions [6]. The biosynthetic origins of lathodoratin and methyl-lathodoratin are completely unknown, though speculations have been put forward [4] (see Scheme 1). Their co-occurrence with isoflavonoids in *Lathyrus* suggests they may arise by degradation of an isoflavone (3-arylchromone) retaining two carbons of the 3-aryl substituent (route a, Scheme 1). Much more attractive is a polyketide origin, with C-2 arising by cyclization of an *O*-methyl group onto the side chain, by an oxidative mechanism as observed during the biosynthesis of rotenoids [7] and 3-benzylchromanones (homoisoflavonoids) [8] (route b). A third possibility (route c) involves the carbon skeleton being built up from three acetate molecules and an isoprene unit. We report here results of investigations on the biosynthetic origins of lathodoratin in *L. odoratus*, which demonstrate that the latter process is indeed the correct one. Furthermore, the isoprene unit acting as a starter for the polyketide chain is derived, not from mevalonate, but from the amino acid isoleucine.

RESULTS AND DISCUSSION

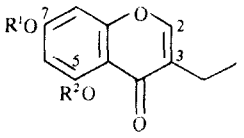
Reference standards of lathodoratin and methyl-lathodoratin were synthesized by the procedure of ref. [9]. Condensation of 2′,4′,6′-trihydroxybutyrophenone with dimethylformamide in the presence of mesyl chloride and boron trifluoride gave excellent yields of lathodoratin (**3**), spectroscopically in agreement with published data [4, 9]. Methylation of the 7-hydroxyl giving methyl-lathodoratin (**4**) was easily achieved using dimethyl sulphate [9]. Indeed, methylation of the chelated 5-hydroxyl could not be performed satisfactorily using normal methylating procedures, and synthesis of dimethyl-lathodoratin (**5**) required the use of methyl iodide in the presence of tetraethylammonium fluoride in HMPT [10].

Phytoalexin synthesis in tissues of *Lathyrus odoratus* was attempted initially using UV light. Earlier experiments [4] had demonstrated that UV light. Earlier experiments [4] had demonstrated that UV light and actinomycin D were effective abiotic inducers of lathodoratin synthesis, although treatment with mercuric chloride gave no response. In another report [5], mercuric



1 R^1 R^2 = OCH_2O, pisatin

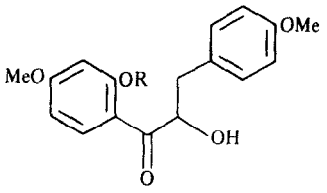
2 R^1 = OMe, R^2 = H, variabilin



3 R^1 = R^2 = H, lathodoratin

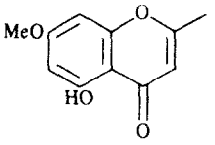
4 R^1 = Me, R^2 = H, methyllathodoratin

5 R^1 = R^2 = OMe, dimethyllathodoratin

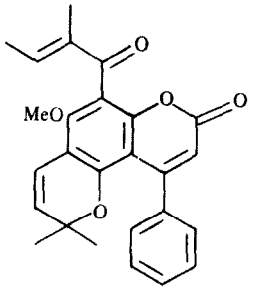


6 R = H, odoratol

7 R = Me, methylodoratol



8 Eugenin



16 Calophyllide

acetate was shown to induce phytoalexin synthesis. In our own studies, irradiation of leaf material with UV light produced no phytoalexins corresponding to lathodoratin or methyl-lathodoratin standards. Mercuric salts were not assessed as inducers, since cellular damage from Hg^{2+} can severely inhibit utilization of labelled materials and hinder biosynthetic investigations [11]. Cupric sulphate induced only traces of lathodoratin when applied to leaf tissue, but was very effective when injected into immature pods of *L. odoratus*. It is highly probable that the lack of success with leaf tissue is a result of poor penetration of the waxy coating on sweet pea leaves. Pod tissue from legumes is, however, often very susceptible to stress, and represents a very convenient vehicle for studies of phytoalexin biosynthesis [11–13]. An aqueous solution of cupric sulphate containing a little Tween was injected into the cavities of young, partially expanded pods of *L. odoratus*. The pods were supported on moist tissue paper in a covered glass tray, then left at 25° in the dark. At intervals, samples were worked-up by homogenization, then extraction with hot ethanol. Extracts were chromatographed using TLC giving material corresponding to authentic standards of lathodoratin, methyl-lathodoratin and pisatin. Concentrations of phytoalexins were

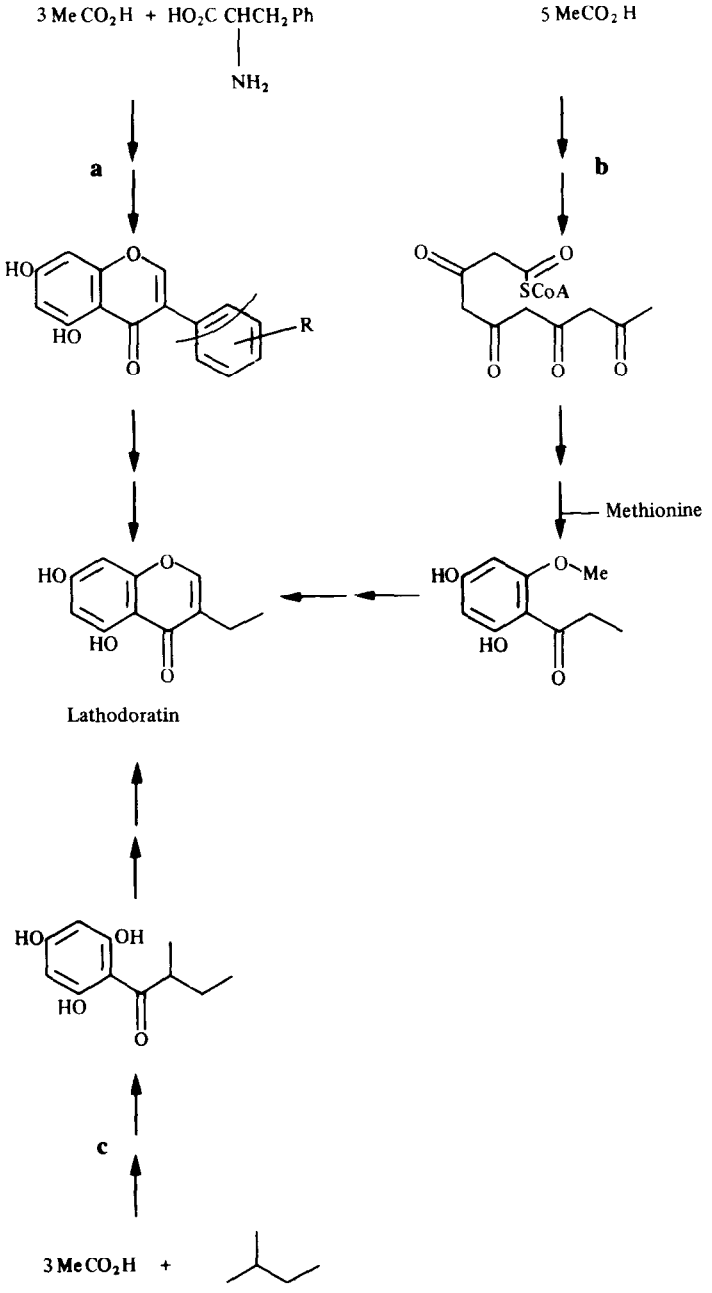
Table 1. Induction of lathodoratin synthesis in *Lathyrus odoratus* pods*

Time after induction (days)	mg lathodoratin isolated
4	0.51
5	0.70
6	0.85
7	0.39
8	0.30

*From 30 g pods, treated with 2% $CuSO_4$/Tween 20.

assessed via UV spectroscopy. Only trace amounts of methyl-lathodoratin were obtained, but lathodoratin and pisatin were efficiently induced by this treatment. Yields of lathodoratin are shown in Table 1. To optimize for lathodoratin production in subsequent feeding experiments, pods were induced for a period of three days, any residual inducer solution was removed by syringe, and a solution of the labelled precursor was then applied instead. Metabolism was allowed to proceed for a further three days.

Preliminary feeding experiments were conducted using sodium [1-^{14}C]acetate, L-[Me-^{14}C]methionine and L-[U-^{14}C]phenylalanine as potential precursors. Although acetate may be expected to be incorporated into lathodoratin by all of the pathways postulated in Scheme 1, methionine would label C-2 if the *O*-methyl cyclization sequence was operative, and [U-^{14}C]phenylalanine would label C-2, C-3, C-4, C-1′ and C-2′ if an isoflavone degradation pathway occurred. Prior to counting, lathodoratin isolated from the feeding experiment was diluted with inactive carrier, then methylated to 7-*O*-methyl-lathodoratin which was purified rigorously to constant specific activity by chromatography and repeated recrystallizations. Pisatin isolated from each experiment was chromatographed repeatedly as in earlier studies [11]. Incorporation data for these experiments are



Scheme 1. Possible biosynthetic pathways to lathodoratin.

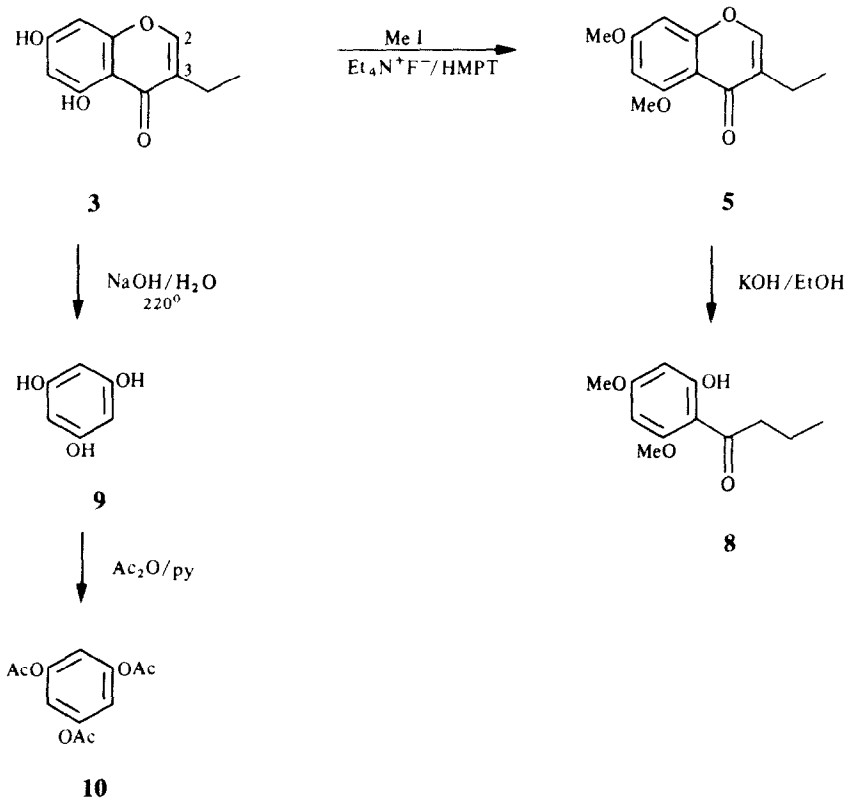
presented in Table 2. All three compounds were incorporated quite efficiently into pisatin as expected, and in agreement with earlier experiments on the biosynthesis of this pterocarpan in *Pisum sativum* [11]. Only acetate was an effective precursor of lathodoratin however. Both methionine and phenylalanine gave very low incorporation levels with high dilution values. Similar results were obtained in a repeat series of experiments.

The labelling pattern in lathodoratin obtained biosynthetically from sodium [1-^{14}C]acetate was investigated by partial degradation as in Scheme 2. Methylation of both hydroxyls gave dimethyl-lathodoratin (**5**), which on base hydrolysis was converted into the butyrophenone (**8**) with loss of C-2. Alkali fusion of lathodoratin gave phloroglucinol (**9**) which was purified as the triacetate (**10**). The relative specific activities of (**5**), (**8**) and (**10**) are presented in Table 3. Within experimental error, these degradation products were all of equal activity. These data show that little, if any, activity from [1-^{14}C]acetate had entered the side-chain fragment, and only the aromatic ring appeared labelled. The failure of methionine and phenylalanine to act as precursors of lathodoratin, coupled with the observed distribution of label from acetate suggests that of the biosynthetic pathways pro-

Table 2. Incorporation of ^{14}C-labelled compounds into phytoalexins in cupric sulphate-treated *L. odoratus* pods*

	Lathodoratin			Pisatin		
Compound fed	mg	% incorporation	Dilution	mg	% incorporation	Dilution
Sodium [1-^{14}C]acetate	1.07	0.10	1110	1.39	0.06	1780
L-[Me-^{14}C]Methionine	1.48	0.001	1.01×10^6	0.33	0.06	4020
L-[U-^{14}C]Phenylalanine	1.30	0.005	3.02×10^6	0.98	0.15	5190
Sodium [1-^{14}C]acetate†	6.40	0.041	2470		Not recorded	
L-[Me-^{14}C]Methionine	1.49	0.003	3.72×10^5	0.75	0.14	2100
L-[U-^{14}C]Phenylalanine	1.80	0.002	3.70×10^6	2.21	0.31	2.22×10^4

*From 30 g pods, except † from 100 g pods.
†Lathodoratin from this experiment used in degradation, Table 3.



Scheme 2. Degradation of lathodoratin.

Table 3. Degradation of lathodoratin derived from sodium [1-^{14}C]acetate

Compound	Specific activity (dpm/mM)	Relative specific activity
Dimethyl-lathodoratin (**5**)	4.33×10^4	1.00
Butyrophenone (**8**)	4.47×10^4	1.03
Triacetylphloroglucinol (**10**)	4.22×10^4	0.97

Table 4. ^{13}C Chemical shift assignments and coupling constants for lathodoratin derived from sodium[$^{13}C_2$]acetate

Carbon	δ_c (ppm)	J_{cc} (Hz)	
2	153.7		
3	125.0		
4	182.7		
4a	105.8	J_{4a-5}	62
5	163.3	J_{5-6}	72
6	99.6	J_{6-7}	68
7	164.8	J_{7-8}	68
8	94.4	J_{8-8a}	74
8a	159.4	J_{8a-4a}	64
1′	18.9	$J_{1'-2'}$	34
2′	13.4		

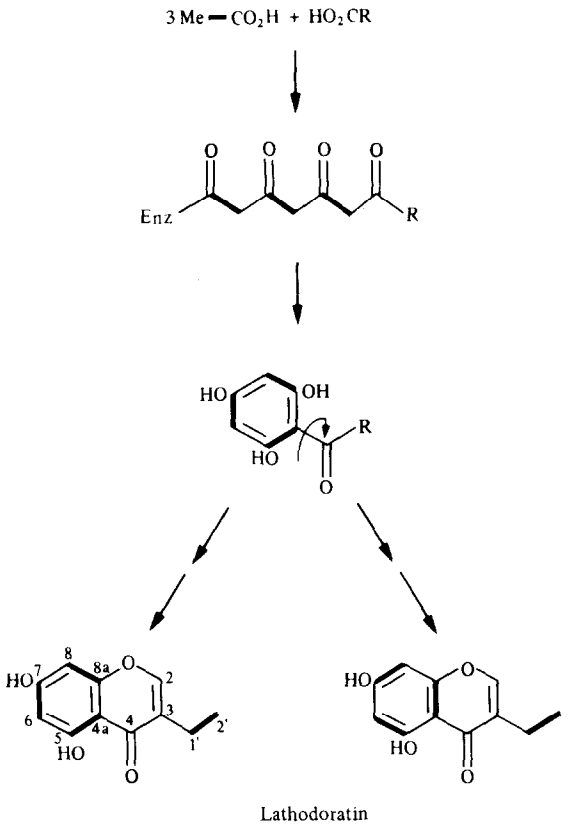
Spectra recorded in CD_3COCD_3 solution with tetramethylsilane internal standard at 100.6 MHz using a Bruker AM 400 spectrometer.

posed in Scheme 1, only the acetate and isoprene unit route c could be operative.

Further information about the labelling pattern in lathodoratin derived from acetate was obtained by feeding sodium [1,2-$^{13}C_2$]acetate and analysis by ^{13}C NMR spectroscopy. Sodium [1,2-$^{13}C_2$]acetate (1 g; 90% ^{13}C at each carbon) together with sodium [1-^{14}C]acetate (50 μCi) were fed to copper sulphate-treated pods (1 kg) using the same techniques as in the small scale experiments. Lathodoratin (37.7 mg; dilution 13.9) and pisatin (17.0 mg; dilution 5.9) were isolated from the extracts. The biosynthesis of pisatin from [$^{13}C_2$]acetate has been reported previously by Stoessl and Stothers [14], who concluded from the observed ^{13}C-satellites that a specific folding of the polyketide chain and reduction of the 'missing' oxygen function prior to ring closure had occurred. In this experiment, our results for pisatin were exactly as ref. [14], demonstrating the incorporation of intact acetate units into C-1a/C-1, C-2/C-3 and C-4/C-4a (enrichment *ca* 2.4% per carbon).

The ^{13}C NMR spectrum of unlabelled lathodoratin was readily interpreted and assigned as in Table 4. In the spectrum of the enriched sample, the six aromatic signals were flanked by satellites. Two of these sets (C-5 and C-8a) were clearly resolved as pairs of satellite signals, whereas those for C-7 and C-4a were unresolved, broad peaks, the result of overlapping satellites having similar coupling constants. The signals for C-6 and C-8 again showed unresolved, overlapping satellites, but because of the higher intensity, and the degree of enrichment, satellites of satellites were also visible, aiding the measurement of coupling constants. The pattern is clearly the result of two types of labelled molecule being present, and can be ascribed to free rotation of the aromatic ring in a biosynthetic intermediate (Scheme 3). The level of enrichment was estimated to be approximately 0.9% at each aromatic carbon. In addition, weaker satellites (*ca* 0.15% enrichment) were observed for the two carbons of the 3-ethyl substituent. No satellites were present for any of the remaining signals. Thus, C-1′/C-2′ also represents an intact acetate unit, but the lower level of enrichment presumably accounts for not detecting this labelling in the ^{14}C-degradation studies.

These results thus demonstrate the polyketide origin of the phloroglucinol ring, and strongly suggest that the remainder of the molecule is a C_5 isoprene unit which acts as a starter (RCO.SCoA, Scheme 3) for chain extension. Mevalonic acid is the usual source of isoprene units, but if mevalonic acid were a precursor of lathodoratin, [$^{13}C_2$]acetate would be expected to provide two intact acetates for the C_5 unit, labelling C-2/C-3 as well as C-1′/C-2′. In some systems, isoprene units are known to have their origins in the amino acids valine, leucine or isoleucine, e.g. the necic acids in *Senecio* alkaloids [15] or tiglic acid in tropane alkaloids [16, 17]. Of these amino acids, isoleucine (**11**) appeared the most promising as a precursor of lathodoratin in that it could provide by transamination and decarboxylation 2-methylbutanoic acid (**12**). This has the correct functionality to make a



Lathodoratin

Scheme 3. Biosynthesis of lathodoratin from [$^{13}C_2$]acetate.

starter for polyketide chain extension and provide the correct carbon skeleton for lathodoratin.

To test this hypothesis, L-[U-^{14}C]isoleucine, L-[U^{14}C]leucine and DL-[4-^{14}C]valine were administered to the *L. odoratus* system. As indicated in Table 5, isoleucine proved the most effective precursor. Naturally, any substrate that could be metabolised to acetylCoA would label lathodoratin via an indirect mechanism and probably at rather lower levels. The incorporation of leucine into sesquiterpenoids has been shown to be via an indirect route involving acetylCoA [18]. This type of mechanism may account for the observed low levels of incorporation of valine and leucine. Incorporation of isoleucine, however, was intact, as demonstrated by degradation studies (Table 6). These showed all the activity to be in the 5-carbon fragment, negligible amounts being detected in the acetate-derived aromatic ring. Approximately one-fifth (22%) of the activity was located at C-2, in accord with a uniformly labelled C_5 unit. Furthermore, the origin of this C_5 unit in lathodoratin from isoleucine is supported by the ^{13}C data. Isoleucine is produced in nature [19] from 2-oxobutanoate and pyruvate, and involving an ethyl migration step [20]. This results in acetate labelling isoleucine (**11**) as shown in Scheme 4, and would yield 2-methylbutanoic acid (**12**) labelled with one intact acetate unit. Its incorporation into lathodoratin would then give the observed labelling pattern (Scheme 4).

Table 6. Degradation of lathodoratin derived from L-[U-^{14}C]-isoleucine

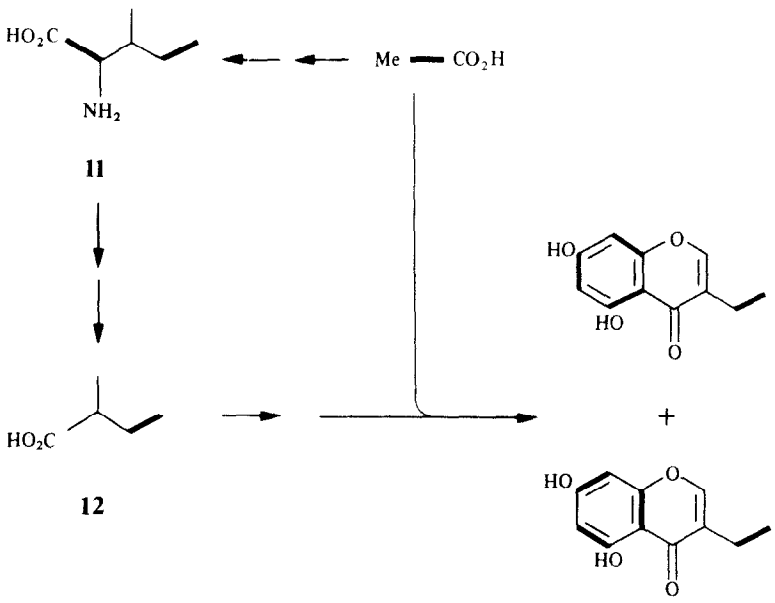
Compound	Specific activity (dpm/mM)	Relative specific activity
Dimethyl-lathodoratin (**5**)	1.43×10^6	1.00
Butyrophenone (**8**)	1.11×10^6	0.78
Triacetylphloroglucinol (**10**)	1.43×10^4	0.01

We propose, therefore, that the biosynthetic pathway to lathodoratin involves isoleucine as precursor, which is metabolised to 2-methylbutanoate as a starter unit for acetate-malonate chain extension (Scheme 5). This may well result in the 2-methylbutyrophenone (**13**) as an intermediate. Formation of the heterocyclic ring then might logically be proposed to follow oxidation of the methyl substituent to the aldehyde oxidation level. An alternative approach, modelled on flavonoid biosynthesis

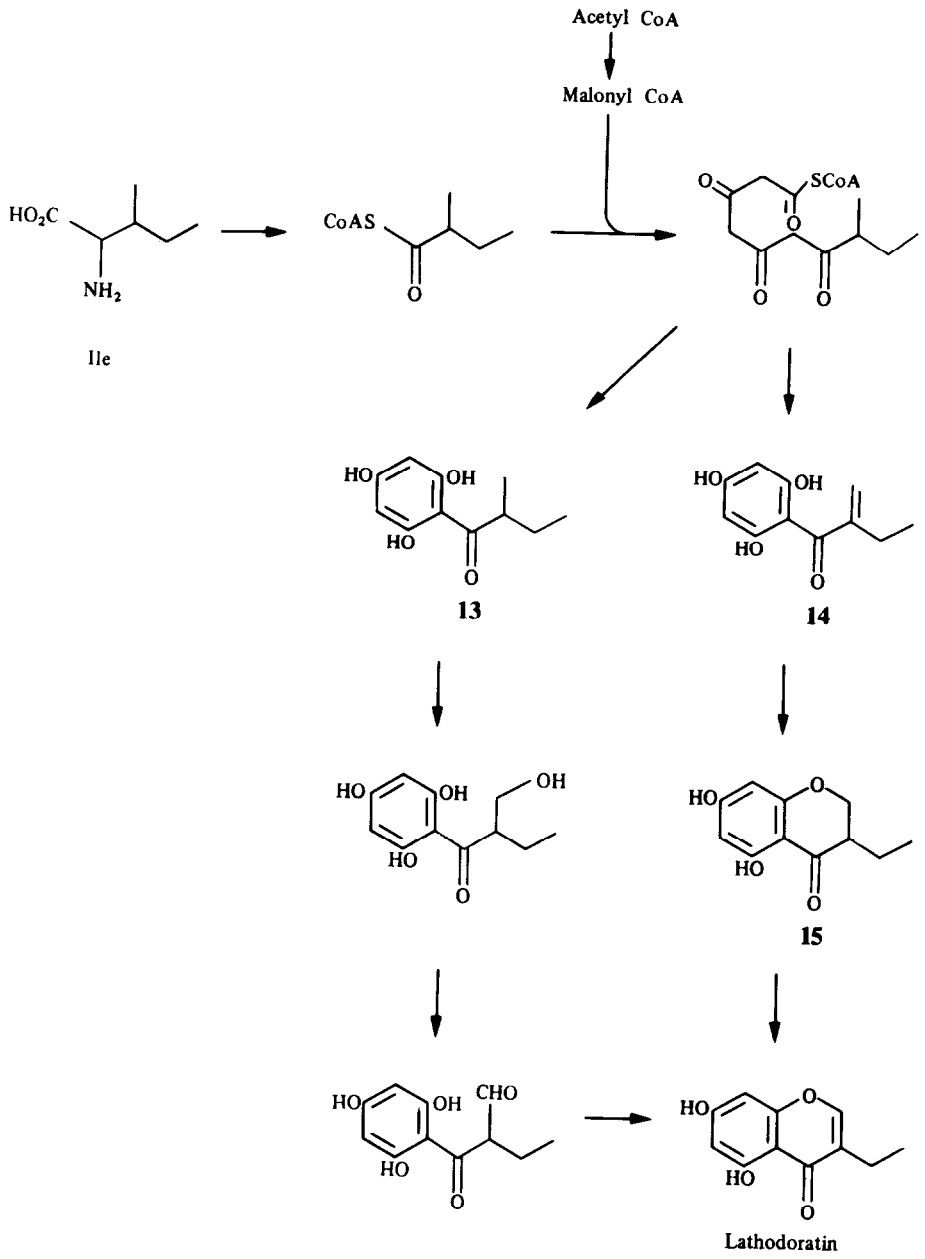
Table 5. Incorporation of ^{14}C-labelled amino acids into lathodoratin in cupric sulphate-treated *L. odoratus* pods*

Compound fed	Lathodoratin produced (mg)	Incorporation (%)	Dilution
L-[U-^{14}C]Isoleucine	1.28	0.90	4.4×10^3
L-[U-^{14}C]Leucine	1.04	0.03	1.0×10^5
DL-[4-^{14}C]Valine	1.73	0.01	7.4×10^3

*30 g pods per experiment.



Scheme 4. Labelling patterns in lathodoratin derived from [$^{13}C_2$]acetate via isoleucine (**11**) and 2-methylbutanoate (**12**).



Scheme 5. Proposed biosynthetic sequences to lathodoratin from isoleucine and acetate–malonate.

[21], would involve cyclization on to an α,β-unsaturated ketone (**14**) followed by oxidation. Intermediates (**14**), (**15**) and lathodoratin would then be analogous to chalcone, flavanone and flavone. However, the exact sequence of events must be speculative only, since no further data are available. Feeding experiments with 2-methylbutanoate and other postulated intermediates may help to clarify the details.

Many organisms are known to use starter molecules other than acetate for chain extension via acetate–malonate. Indeed, in plants, flavonoids are formed by malonate chain extension of a cinnamoyl-CoA starter [21], and subsequent modification including an aryl migration may then lead to isoflavonoids such as pisatin [22]. However, the participation of isoleucine as source of the starter unit is unusual, and we know of few other examples. Isoleucine, via 2-methylbutanoyl-CoA and 2-methylbutanoyl-ACP, provides the starter unit for biosynthesis of the fatty acids 12-methyltetradecanoic and 14-methylhexadecanoic acids in *Curtobacterium pusillum* [23]. In the plant *Calophyllum inophyllum*, the neoflavonoid calophyllide (**16**) contains a tigloyl-phloroglucinol unit which probably arises from a sequence similar to that proposed for lathodoratin. The tigloyl group has been demonstrated to be derived specifically from isoleucine, probably via 2-methylbutanoate [24]. The bio-

synthetic pathway to lathodoratin bears little similarity to that to pisatin, except in the formation of an aromatic ring from acetate. Even then, a specific reduction step resulting in loss of an oxygen function occurs during formation of pisatin, though not for lathodoratin. Why *L. odoratus* should produce two major phytoalexins via unrelated biosynthetic sequences is an intriguing problem.

Earlier studies of the phytoalexin response of *Lathyrus odoratus* have reported the formation of lathodoratin [4, 5] methyl-lathodoratin [4, 5], pisatin [3–5], traces of variabilin (**2**) [3], odoratol (**6**) [5] and methylodoratol (**7**) [5]. In our work, only lathodoratin and pisatin were produced in any quantity. Efforts were made to isolate these other metabolites from the large-scale [$^{13}C_2$]acetate feeding experiment. This batch (1 kg) of cupric sulphate-induced pods, yielded lathodoratin (37.7 mg), methyl-lathodoratin (1.76 mg) and pisatin (17.0 mg). Variabilin in most TLC systems co-chromatographs with pisatin, but NMR spectra of the pisatin isolated here showed no traces of variabilin. A minor chromatographic band isolated had UV characteristics (λ_{max} 225, 275, 307 nm) similar to those reported for methylodoratol (λ_{max} 226, 272, 305 nm [5]), but only traces of this material were present. A further metabolite isolated in small yield (0.16 mg) was shown to be identical (co-chromatography, UV, MS) to 5,7-dimethyl-lathodoratin (**5**) synthesized previously during the degradative studies. This has not been reported previously as a natural product. In a TLC bioassay using *Cladosporium herbarum* [25], dimethyl-lathodoratin proved considerably more fungitoxic than either lathodoratin or methyl-lathodoratin.

EXPERIMENTAL

TLC. TLC was carried out using 0.5 mm layers of silica gel (Merck TLC–Kiesel gel $60GF_{254}$). Analar acetone was used for elution of TLC zones.

Labelled compounds. Sodium [1-^{14}C]acetate (2.4 mCi/mM), L-[Me-^{14}C]methionine (60.4 mCi/mM), L-[U-^{14}C]phenylalanine (504 mCi/mM), L-[U-^{14}C]isoleucine (337 mCi/mM), L-[U-^{14}C]leucine (348 mCi/mM), DL-[4-^{14}C]valine (4.8 mCi/mM), and sodium [1,2-$^{13}C_2$]acetate (90% ^{13}C at each carbon) were purchased (Amersham).

2′,4′,6′-Trihydroxybutyrophenone. Dry phloroglucinol (5 g; purified by vacuum sublimation), freshly distilled *n*-butyronitrile (4 g), dry ether (75 ml) and dry $ZnCl_2$ (2 g) were stirred together in a 2-necked flask cooled in an ice-bath. Dry HCl gas was passed through the cooled, stirred mixture for approximately 4 hr, during which time an orange ppt. formed. The flask was stoppered and kept at 0° for 24 hr. The supernatant liquid was decanted off, the ppt. washed with ether (×2) then aqueous NaOH (10%) added to bring the pH to 6.5–7. The mixture was diluted with H_2O (100 ml), then heated under reflux for 1 hr and filtered hot. The filtrate on cooling gave 2′,4′,6′-trihydroxybutyrophenone, which was recrystallized from H_2O. Yield 50 g (64%), mp 176° (lit. [26] 183°). ^{1}H NMR (90 MHz, acetone-d_6, TMS): δ0.95 (3H, *t*, J = 7 Hz, $-CH_2CH_2\underline{Me}$), 1.65 (2H, *sextet*, J = 7 Hz, $-CH_2\underline{CH_2}CH_3$), 3.05 (2H, *t*, J = 7 Hz, $-\underline{CH_2}CH_2CH_3$), 5.95 (2H, *s*, H-3′, H-5′).

5,7-Dihydroxy-3-ethylchromone (lathodoratin) (**3**). A solution of 2′,4′,6′-trihydroxybutyrophenone (5.3 g) in dry DMF (150 ml) was treated slowly with BF_3-etherate (15.5 g) in a 2-necked flask cooled in an ice-bath. After the exothermic reaction had ceased, a solution of methanesulphonyl chloride (9.4 g) in dry DMF (150 ml) at 50° was added. The mixture was then heated on a steam bath in an open beaker for 1.5 hr. The cooled product was poured, with stirring, into water (500 ml), then extracted with EtOAc (3 × 250 ml). The combined extracts were washed with H_2O (5 × 100 ml), dried over $MgSO_4$, and evapd to dryness. The product was recrystallized from MeOH to give lathodoratin (4.5 g, 81%), mp 205–206° (lit [9] 201.5–202.5°). UV λ_{max}^{EtOH} nm: 208 (log ε 4.38), 228 (4.24), 250 (4.29), 258 (4.37), 295 (3.84), 322 sh (3.74). ^{1}H NMR (90 MHz, acetone-d_6, TMS): δ1.15 (3H, *t*, J = 7 Hz, $-CH_2\underline{Me}$), 2.40 (2H, *q*, J = 7 Hz, $-\underline{CH_2}Me$), 2.8 (1H, *br*, 7-OH), 6.20 and 6.30 (each 1H, *d*, J = 1.5 Hz, H-6,8), 7.90 (1H, *s*, H-2), 12.95 (1H, *s*, 5-OH).

5-Hydroxy-7-methoxy-3-ethylchromone (methyl-lathodoratin) (**4**). Lathodoratin (30 mg) in dry DMF (5 ml) was stirred and heated under reflux with dry K_2CO_3 (1 g) and Me_2SO_4 (68 μl) for 1.5 hr. The mixture was cooled, poured into H_2O and extracted with EtOAc (3 × 10 ml). The combined extracts were washed with H_2O (3 × 10 ml), dried over $MgSO_4$, and evapd to dryness. The product was purified by TLC (hexane–Me_2CO, 2:1) then recrystallized from aq. EtOH to give methyl-lathodoratin (22.5 mg, 70%), mp 69–70° (lit [9] 68–69.5°). UV λ_{max}^{EtOH} nm: 208 (log ε 4.32), 229 (4.23), 250 (4.30), 257 (4.32), 295 (3.92), 322 sh (3.63). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ1.15 (3H, *t*, J = 7 Hz, $-CH_2\underline{Me}$), 2.45 (2H, *q*, J = 7 Hz, $-\underline{CH_2}Me$), 3.85 (3H, *s*, OMe), 6.40 (2H, *s*, H-6, H-8), 7.70 (1H, *s*, H-2), 12.80 (1H, *s*, 5-OH).

5,7-Dimethoxy-3-ethylchromone (dimethyl-lathodoratin) (**5**). Tetraethylammonium fluoride (48 mg) was dried *in vacuo* over P_2O_5 at 60° for 12 hr. This was added to a solution of dry lathodoratin (100 mg) in dry HMPT (1 ml), and the mixture was kept at 60° under vacuum (rotary evaporator) for 10 min. Methyl iodide (206 mg) in dry HMPT (1.4 ml) was then added to the mixture, and the solution was stoppered and stirred at 60° for 3 hr. Ether (20 ml) and H_2O (10 ml) were added to the reaction mixture which was shaken vigorously, and the layers separated. The aqueous layer was extracted further with ether (3 × 50 ml), and the combined ether layers were washed with H_2O (3 × 50 ml), dried over $MgSO_4$ and evapd to dryness. The residual solid was purified by TLC (hexane–Me_2CO, 2:1) to give dimethyl-lathodoratin, which was recrystallized from aq. EtOH. Yield 70 mg (62%), mp 99–100°. UV λ_{max}^{EtOH} nm: 207 (log ε 4.27), 228 (4.38), 245 (4.30), 253 (4.33), 283 (3.95), 305 sh (3.80). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ1.15 (3H, *t*, J = 7 Hz, $-CH_2\underline{Me}$), 2.45 (2H, *q*, J = 7 Hz, $-\underline{CH_2}Me$), 3.85 and 3.93 (each 3H, *s*, OMe), 6.35 and 6.40 (each 1H, *d*, J = 2 Hz, H-6,8), 7.55 (1H, *s*, H-2). EIMS (probe) 70 eV, *m/z* (rel. int.): 234 [M$^+$] (100%), 233 (49), 215 (27), 205 (29), 203 (33), 188 (18).

Plant material, feeding experiments and isolation of phytoalexins. Lathyrus odoratus (sweet pea, Spencer varieties mixed, Suttons Seeds Ltd, Torquay) plants were grown under normal garden conditions. Young, partially expanded pods (30 g) were excised and the pod cavities were filled with 2% aqueous $CuSO_4$ solution containing a few drops of Tween 20. The pods were filled using a syringe, with a second syringe needle as a vent, using approximately 10 ml $CuSO_4$ solution per 30 g pods. The pods were placed on moist tissue paper in glass trays, the trays were then covered and placed in a growth cabinet at 25° in the dark for 72 hr. Any remaining $CuSO_4$ was then removed by syringe, and a solution of the labelled precursor (10–50 μCi) in H_2O (3 ml) was distributed evenly amongst the pods. The pods were returned to the growth cabinet for a further 72 hr. The pods were then worked up by homogenizing in a blender with hot EtOH (100 ml). The slurry was filtered, and the solids re-extracted with further hot EtOH (4 × 100 ml), the combined extracts then being evapd to about 5 ml. Water (25 ml) was added to the residue, and the mixture was extracted with EtOAc (100 ml, then 4 × 50 ml). The combined extracts were evapd to dryness. The residue was separated by TLC ($CHCl_3$–MeOH, 50:1) and a band

corresponding to authentic markers of both lathodoratin and pisatin was eluted. The two compounds were separated by further TLC using solvents hexane–EtOAc–MeOH, 60:40:1 and then hexane–Me_2CO, 2:1. Their concns were assessed by UV absorption of EtOH solutions, lathodoratin λ_{max} 258 nm (log ε4.37) and pisatin λ_{max}307 nm (log ε 3.86) [27].

Lathodoratin was diluted with inactive carrier (30 mg), then methylated to methyl-lathodoratin as described above. The methyl ether was purified to constant specific activity by TLC (hexane–Me_2CO, 2:1 and $CHCl_3$–MeOH, 25:1) and repeated recrystallization from aqueous EtOH. Pisatin was purified to constant specific activity by further TLC as described earlier [11].

In a large-scale experiment, $CuSO_4$-induced pods (1 kg) yielded lathodoratin (37.7 mg) and pisatin (17.0 mg) by the procedures given above. Further chromatographic bands corresponding to authentic markers of methyl-lathodoratin and dimethyl-lathodoratin were eluted and purified further by TLC using solvents hexane–Me_2CO, 2:1 and then hexane–EtOAc–MeOH, 60:40:1. Methyl-lathodoratin (1.76 mg) and dimethyl-lathodoratin (0.16 mg) were isolated, and their identity confirmed by UV, NMR and MS measurements. A chromatographic band running just in front of pisatin, presumed to contain odoratol/methylodoratol [5] was examined further and resolved into three bands by TLC in hexane–EtOAc–MeOH (60:40:1). One of these bands had UV absorbances (λ_{max}^{EtOH} nm: 225, 275, 307) corresponding to those reported for methylodoratol (226, 272, 305 nm) [5], and was tentatively identified as this compound. Its concentration was estimated at 0.12 mg, based on log $\varepsilon = 4.18$ at 276 nm for dihydrochalcones [28].

Degradation of lathodoratin. A mixture of dimethyl-lathodoratin (45 mg) and KOH (300 mg) in 50% aqueous EtOH (8 ml) was stirred and heated under reflux for 3 hr. The mixture was cooled, neutralized with dilute HCl and extracted with EtOAc (3 × 10 ml). The combined extracts were dried over $MgSO_4$ and evapd to dryness. The product was purified by TLC (hexane–Me_2CO, 2:1 and toluene–EtOAc–MeOH, 60:40:1) to give 4′,6′-dimethoxy-2′-hydroxybutyrophenone (**8**), which was recrystallized (2x) from hexane. Yield 22 mg, mp 70–71°, lit [26] 70°. ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ1.10, (3H, *t*, $J = 7$ Hz, $-CH_2CH_2\underline{Me}$), 1.70 (2H, *sextet*, $J = 7$ Hz, $-CH_2\underline{CH_2}Me$), 2.95 (2H, *t*, $J = 7$ Hz, $-\underline{CH_2}CH_2Me$), 3.85 and 3.90 (each 3H, *s*, OMe), 5.95 and 6.10 (each 1H, *d*, $J = 2$ Hz, H-6,8).

A mixture of lathodoratin (50 mg), NaOH (250 mg) and H_2O (1.5 ml) was stirred and heated at 220°C for 45 min. The mixture was cooled, neutralized with dilute HCl and extracted with ether (3 × 10 ml). The combined extracts were dried over $MgSO_4$ then evapd to dryness. The residue was purified by TLC (toluene–HCO_2Et–HCO_2H, 5:4:1 and $CHCl_3$–MeOH, 25:1) to give phloroglucinol (25 mg), mp 215–216°, lit [29] 217–219°. This was acetylated (pyridine–Ac_2O) to give triacetylphloroglucinol (**10**), which was purified by TLC (hexane–Me_2CO, 2:1) and recrystallization (× 2) from aq. EtOH. Yield 20 mg, mp 105–106°, lit [29] 104°.

REFERENCES

1. Bailey, J. A. and Mansfield, J. W. (eds) (1982) *Phytoalexins* Blackie, Glasgow.
2. Ingham, J. L. (1982) in *Phytoalexins* (Bailey, J. A. and Mansfield, J. W., eds) p. 21, Blackie, Glasgow.
3. Robeson, D. J. and Harborne, J. B. (1980) *Phytochemistry* **19**, 2359.
4. Robeson, D. J., Ingham, J. L. and Harborne, J. B. (1980) *Phytochemistry* **19**, 2171.
5. Fuchs, A. De Vries, F. W., Landheer, C. A. and Van Veldhuizen, A. (1984) *Phytochemistry* **23**, 2199.
6. Stoessl, A. and Stothers, J. B. (1978) *Can. J. Botany* **56**, 2589.
7. Crombie, L., Dewick, P. M. and Whiting, D. A. (1973) *J. Chem. Soc., Perkin Trans. I* 1285.
8. Dewick, P. M. (1985) *Phytochemistry* **14**, 983.
9. Clough, J. M. and Snell, B. K. (1981) *Phytochemistry* **20**, 1752.
10. Picq, M., Prigent, A. F., Chabannes, B., Pacheco, H., Parent, P. and Pichat, L. (1984) *Tetrahedron Letters* **25**, 2227.
11. Banks, S. W. and Dewick, P. M. (1982) *Phytochemistry* **21**, 2235.
12. Banks, S. W. and Dewick, P. M. (1983) *Phytochemistry* **22**, 2729.
13. Al-Ani, H. A. M. and Dewick, P. M. (1985) *Phytochemistry* **24**, 55.
14. Stoessl, A. and Stothers, J. B. (1979) *Z. Naturforsch.* **34c**, 87.
15. Robins, D. J. (1982) *Prog. Chem. Org. Nat. Prod.* **41**, 115.
16. Basey, K. and Woolley, J. G. (1973) *Phytochemistry* **12**, 2197.
17. Beresford, P. J. and Woolley, J. G. (1974) *Phytochemistry* **13**, 2143.
18. Anastasis, P., Freer, I., Picken, D., Overton, K., Sadler, I. and Singh, S. B. (1983) *J. Chem. Soc., Chem. Commun.* 1189.
19. Zubay, G. (1983) in *Biochemistry*, p. 848, Addison–Wesley, Reading, MA.
20. Cahill, R., Crout, D. H. G., Gregorio, M. V. M., Mitchell, M. B. and Muller, U.S. (1983) *J. Chem. Soc., Perkin Trans. I.* 173.
21. Ebel, J. and Hahlbrock, K. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 641. Chapman & Hall, London.
22. Dewick, P. M. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 535. Chapman & Hall, London.
23. Kawaguchi, A., Uemura, N. and Okuda, S. (1986) *J. Biochem. (Tokyo)* **99**, 1735.
24. Kunesch, G. and Polonsky, J. (1969) *Phytochemistry* **8**, 1221.
25. Ingham, J. L. (1976) *Phytopath. Z.* **87**, 353.
26. Canter, F. W., Curd, F. H. and Robertson, A. (1931) *J. Chem. Soc.* 1245.
27. Perrin, D. R. and Bottomley, W. (1962) *J. Am. Chem. Soc.* **84**, 1919.
28. Jensen, S. R., Nielsen, B. J. and Norn, V. (1977) *Phytochemistry* **16**, 2036.
29. Dictionary of Organic Compounds, 4th Edn (1965) Eyre and Spottiswoode, London.