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# BIOSYNTHESIS OF THE 3-BENZYLCHROMAN-4-ONE EUCOMIN IN *EUCOMIS BICOLOR*

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**Key Word Index**—*Eucomis bicolor*; Liliaceae; eucomin; 3-benzylchroman-4-one; homoisoflavonoid; biosynthesis; chalcone.

**Abstract**—Feeding experiments have demonstrated the specific incorporation of radioactivity from DL-phenylalanine-[1-$^{14}$C], L-phenylalanine-[U-$^{14}$C], sodium acetate-[2-$^{14}$C] and L-methionine-[*methyl*-$^{14}$C] into the 3-benzylchroman-4-one eucomin in *Eucomis bicolor*. The labelling patterns indicate that eucomin is biosynthesized by the addition of a carbon atom derived from methionine onto a $C_{15}$ chalcone-type skeleton. Radioactivity from 2′,4′,4-trihydroxy-6′-methoxychalcone-[*methyl*-$^{14}$C] and 2′,4′-dihydroxy-4,6′-dimethoxychalcone-[6′-*methyl*-$^{14}$C] was incorporated into eucomin, the latter compound being the better precursor, demonstrating the feasibility that 2′-methoxychalcones are biosynthetic precursors of the "homoisoflavonoids". Possible biosynthetic relationships in this class of compounds are discussed.

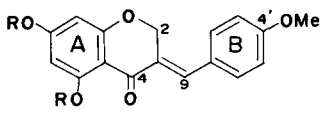
## INTRODUCTION

A number of phenolic compounds containing a basic 3-benzylchroman-4-one skeleton have been isolated from species of *Eucomis* [1–4] and *Scilla* [5] (Liliaceae). This skeleton differs from that of an isoflavonoid by the presence of an extra carbon atom, and the name "homoisoflavonoid" has been proposed for compounds of this type. This paper reports the results of feeding experiments performed to investigate the biosynthesis of 5,7-dihydroxy-3-(4-methoxybenzal)-chroman-4-one, eucomin (**1**) in *Eucomis bicolor* Bak. A preliminary communication [6] has been published.

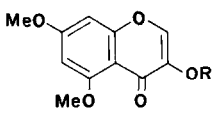
## RESULTS AND DISCUSSION

Preliminary experiments to ascertain suitable conditions for feeding showed that the eucomin content of the plant *E. bicolor* was at a maximum in the dormant bulb, decreasing as growth progressed and the flower spike was produced. The content then increased slowly during the fruiting period towards its maximum again in the dormant bulb. Eucomin appeared to be present only in the bulb and root portion of the plant and existed in the free state, no further material was liberated on acid hydrolysis.

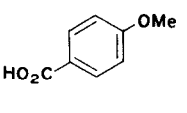
Radioactive precursors fed in aqueous solution via the roots were readily absorbed by the plant; injection procedures proved less satisfactory. The labelled compounds tested for precursor efficiency were DL-phenylalanine-[1-$^{14}$C], L-phenylalanine-[U-$^{14}$C], L-methionine-[*methyl*-$^{14}$C] and sodium acetate-[2-$^{14}$C], and these were administered to plants in the fruiting period of development for feeding times of 48 and 72 hr. Eucomin was isolated from the bulb and roots by standard procedures (see Experimental) and purified to con-



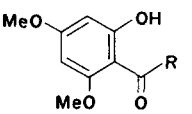

(**1**) R = H
(**2**) R = Me




(**3**) R = H
(**4**) R = Me




(**5**)




(**6**) R = OH
(**7**) R = $CH_2OMe$

Table 1. Incorporation of precursors into eucomin in *Eucomis bicolor*, and relative specific activities of degradation products

| Compound fed | Specific activity of eucomin (dpm/mM) | Incorporation (%) | Relative specific activities | | | |
|---|---|---|---|---|---|---|
| | | | Dimethyl-eucomin | Anisic acid | $MeEt_3N^+I^-$ (4'-OMe) | Dimethoxy-salicyclic acid |
| DL-Phenylalanine-[1-$^{14}$C]* | $6{\cdot}80 \times 10^4$ | 0·0022 | 1·00 | 0·02 | | 1·00‡ |
| L-Phenylalanine-[U-$^{14}$C]* | $2{\cdot}67 \times 10^4$ | 0·0010 | 1·00 | 0·73 | | 0·14 |
| Sodium acetate-[2-$^{14}$C]* | $9{\cdot}20 \times 10^3$ | 0·0002 | 1·00 | 0·08 | | 0·91 |
| L-Methionine-[*methyl*-$^{14}$C]* | $2{\cdot}37 \times 10^4$ | 0·0005 | 1·00 | 0·46 | | 0·06 |
| L-Methionine-[*methyl*-$^{14}$C]† | $4{\cdot}71 \times 10^4$ | 0·0014 | 1·00§ | 0·40 | 0·38 | |

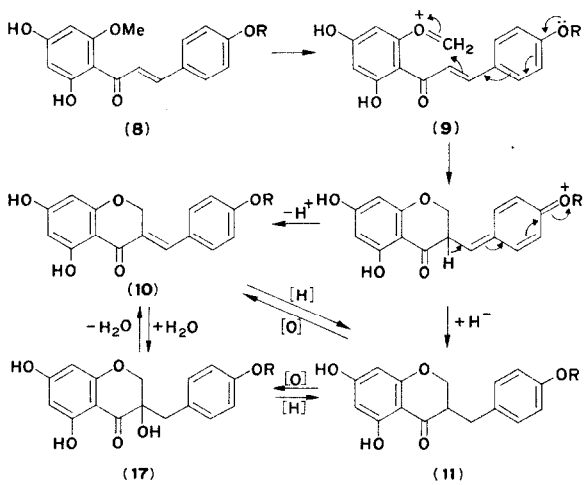
* Feeding period 72 hr. † Feeding period 48 hr. ‡ Relative sp. act. of $BaCO_3$ (C-4) was 0·92. § Relative sp. act. of 2'-hydroxy-2,4',6'-trimethoxyacetophenone was 0·07.

stant specific activity as the dimethyl ether (**2**). The incorporation of radioactivity was low (Table 1) but of sufficient magnitude to permit degradative studies and provide a reasonably accurate assessment of the labelling pattern produced by these precursors.

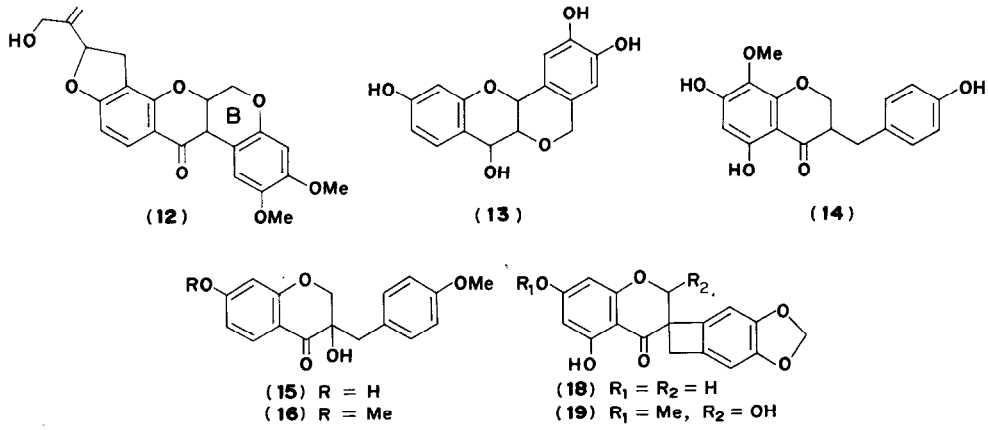
Degradation of the labelled dimethyl ether (**2**) by $OsO_4$–$KClO_3$ cleavage liberated anisaldehyde and 3-hydroxy-5,7-dimethoxychromone (**3**). Anisaldehyde was oxidized to anisic acid (**5**) using alkaline $KMmO_4$; the chromone was either treated with alkaline $H_2O_2$ to yield 4,6-dimethoxysalicylic acid (**6**), or, after methylation to 3,5,7-trimethoxychromone (**4**), hydrolysed to give 2'-hydroxy-2,4',6'-trimethoxyacetophenone (**7**). The two aromatic acids were degraded further and the relative specific activities of the degradation products are presented in Table 1.

The results indicate that, within the limits of experimental error, radioactivity from phenylalanine-[1-$^{14}$C] was entirely localized at C-4 of eucomin. The corresponding experiment using phenylalanine-[U-$^{14}$C] showed that 73% of the radioactivity was recovered in anisic acid, and a further 14% was present in 4,6-dimethoxysalicylic acid (presumably all located in the carboxyl). These figures approximate well to the expected 78% and 11% respectively if labelling of the phenylalanine precursor is indeed completely uniform. These two experiments indicate that a $C_6C_3$ unit derived from phenylalanine is incorporated intact into the eucomin molecule, becoming C-4, C-3, C-9, and the aromatic ring B. The *O*-methyl on ring B is supplied by methionine, and since acetophenone (**7**) was virtually inactive, it is concluded that methionine also acts as the source of C-2. The aromatic ring A is acetate-derived.

The labelling patterns in the eucomin skeleton from these precursors are analogous to those demonstrated in the biosynthesis of flavonoid compounds [7], but differ in that an extra carbon atom derived from methionine is added to produce the heterocyclic ring. It is well established [7] that the fundamental $C_{15}$ skeleton of flavonoids is provided by the chalcones, and these results offer support for the proposed [4, 8] involvement of 2'-methoxychalcones in "homoisoflavonoid" biosynthesis (Scheme 1). It is envisaged that cyclisation of intermediate (**9**), produced from chalcone (**8**) by oxidation of the 2'-methoxyl, would produce either 3-benzalchroman-4-one (**10**) by loss of a proton, or 3-benzylchroman-4-one (**11**) by addition of a hydride ion. The alternative hypothesis [4] in which C-9 is the "extra" carbon atom must now be excluded. The utilisation of an *O*-methyl group to form a carbon–carbon linkage in flavonoid-de-




Scheme 1. Possible biosynthetic routes from 2'-methoxychalcones to 3-benzylchroman-4-one derivatives.




rived compounds has been demonstrated [9] in the biosynthesis of the B-ring of rotenoids e.g. amorphigenin (**12**), from 2′-*O*-methyl isoflavones, and *O*-methyl groups are also believed to be involved in the formation of peltogynoids [10], e.g. peltogynol (**13**), by analogy with *in vitro* processes. The similar cyclisation of an *N*-methyl group to give a "berberine bridge" is well established in alkaloid biosynthesis [11].

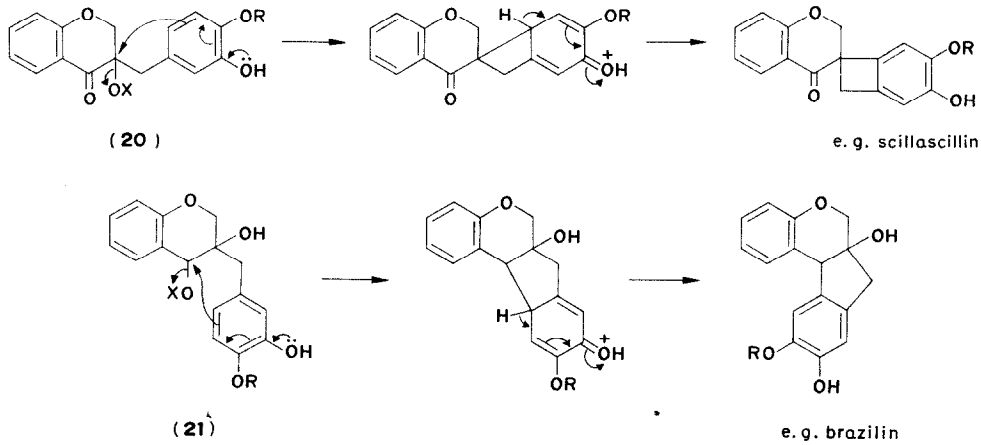
To test the proposal, two specifically-labelled chalcones, 2′,4′,4-trihydroxy-6′-methoxychalcone-[*methyl*-$^{14}$C] (**8**, R=H) and 2′,4′-dihydroxy-4,6′-dimethoxychalcone-[6′-*methyl*-$^{14}$C] (**8**, R=Me) were prepared. These 2 compounds were synthesized via base condensation of the appropriate aldehyde and 2-*O*-methylphoracetophenone-[*O*-*methyl*-$^{14}$C], which was obtained by partial methylation of 4-*O*-benzoylphloracetophenone with dimethyl sulphate-[$^{14}$C] followed by base hydrolysis. The two chalcones were fed as their Na salts via the roots to *E. bicolor* for a period of 72 hr. The eucomin isolated was purified as before, and the incorporation data are presented in Table 2. Once again, incorporation of radioactivity was poor, and unfortunately did not permit degradation and determination of the specificity of labelling. The low incorporations do not necessarily imply a random incorporation of radioactivity. The earlier results show that very low incorporations can be entirely specific, and the low figures obtained here presumably reflect the low rate of synthesis of eucomin in *E. bicolor*. Determination of the specificity of labelling will require the use of chalcones of higher specific activity. It would appear, however, that these 2′-methoxychalcones can be converted into eucomin in *E. bicolor*, the dihydroxydimethoxychalcone being the better precursor, and the results demonstrate the feasibility of the initial transformations in Scheme 2 (R=Me).

These feeding experiments establish that eucomin, and presumably other compounds containing the 3-benzylchroman-4-one skeleton, are biosynthesised by modification of the $C_6.C_3.C_6$ chalcone–flavonoid skeleton, by insertion of an extra carbon atom. The name "homoisoflavonoid" is thus inappropriate and misleading, since isoflavonoid compounds are formed in nature by a pathway involving a characteristic 2,3-aryl migration step [7]. No such rearrangement occurs during the biosynthesis of eucomin, and the "homoisoflavonoids" merely represent a further modification of the unrearranged flavonoid-type skeleton. The systematic name 3-benzylchroman-4-one is thus preferable.

Table 2. Incorporation of labelled 2′-methoxychalcones into eucomin in *Eucomis bicolor*

| Chalcone fed* | Specific activity of eucomin (dpm/mM) | Incorporation (%) | Dilution of sp. act. |
|---|---|---|---|
| 2′,4′,4-Trihydroxy-6′-methoxychalcone-[*methyl*-$^{14}$C] | $4{\cdot}68 \times 10^3$ | 0·0009 | $4{\cdot}38 \times 10^4$ |
| 2′,4′-Dihydroxy-4,6′-dimethoxychalcone-[6′-*methyl*-$^{14}$C] | $2{\cdot}03 \times 10^4$ | 0·0050 | $1{\cdot}00 \times 10^4$ |

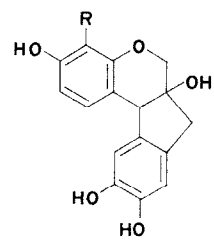
* Feeding period 72 hr.




Scheme 2. Hypothetical biosynthetic routes to scillascillin- and brazilin-like compounds by cyclization of 3-benzylchroman-4-one derivatives.

The relationship of 3-benzalchroman-4-ones such as eucomin to other variants such as the 3-benzylchroman-4-ones (e.g. 3,9-dihydropunctatin [3], **14**) and 3-hydroxy-3-benzylchroman-4-ones (e.g. eucomol [1], **15**) is not yet established. Possible biosynthetic routes are depicted in Scheme 1. Tamm has suggested [4] that $\Delta^{3,9}$ compounds arise via the 3-hydroxy derivatives from 3,9-dihydro compounds (Scheme 1, **11** → **17** → **10**), since the feeding of sodium acetate-[1-$^{14}$C] to *E. bicolor* produced a higher incorporation of radioactivity into eucomol (**15**) than into eucomin (**1**). (*E. bicolor* bulbs produce three main phenolic compounds [1, 4], eucomin, eucomol and 7-*O*-methyleucomol, **16**). Only low levels of radioactivity were obtained, and such conclusions can only be speculative. Scheme 1 includes formation from intermediate (**9**) of either 3-benzalchroman-4-one (**10**) or 3-benzylchroman-4-one (**11**). The latter reaction sequence would represent no overall change in oxidation level from methoxychalcone (**8**) to 3-benzylchroman-4-one (**11**). A radical version of the scheme may also be written. 3-Hydroxy-derivatives (**17**) may then be produced by hydration of (**10**) or hydroxylation of (**11**). Interconversions of (**10**) and (**11**) are also possible.

The recent report [5] of 3-benzylchroman-4-one derivatives occurring in *Scilla scilloides* is interesting in that a new variation on this skeleton has been identified. Two compounds scillascillin (**18**) and 2-hydroxy-7-*O*-methylscillascillin (**19**) are characterized by a 3-*spiro*-cyclobutene ring. The origin of this structure is unknown, but it may be derived from a suitably activated 3-hydroxy-3-benzylchroman-4-one (**20**) by the cyclisation sequence shown in Scheme 2. A related cyclisation of the activated 3-hydroxy-3-benzylchroman-4-ol (**21**), this time to produce a cyclopentene ring, would explain the origin of brazilin (**22**) and haematoxylin (**23**) obtained from the heartwoods of *Caesalpinia* spp. and *Haematoxylon campechianum* (Leguminosae) respectively. These structures have promoted a number of hypotheses [12] regarding their formation in nature, and their probable origin from the 3-benzylchroman-4-one skeleton, by processes which roughly parallel synthetic routes [13] to these compounds, appears the most attractive. So far there are no reports of simple 3-benzylchroman-4-one derivatives occurring outside of the Liliaceae.




## EXPERIMENTAL

*General.* Bulbs of *Eucomis bicolor* were obtained from Messrs. Thompson and Morgan (Ipswich) Ltd., and Walter Blom & Son Ltd., Leavesden. UV spectra were measured in EtOH. TLC was carried out using 0·5 mm layers of Si gel (Merck Kieselgel

$GF_{254}$) and the solvent systems: (A) $C_6H_6$–EtOAc–MeOH–petrol (60–80°), 6:4:1:8; (B) toluene–$HCO_2Et$–$HCO_2H$, 5:4:1; (C) $CHCl_3$–*iso*-PrOH, 10:1; (D) $C_6H_6$–EtOAc–MeOH–petrol (60–80°), 6:4:1:3; (E) $C_6H_6$–EtOH, 92:8. Radioactive samples were counted in a dioxan-based scintillator solution, or, in the case of $BaCO_3$ samples in thixotropic gels (Cab-O-Sil). Efficiencies were measured by means of toluene-[$^{14}$C] internal standards.

*Radiochemicals.* DL-Phenylalanine-[1-$^{14}$C] (sp. act. 59 mCi/mM), L-phenylalanine-[U-$^{14}$C] (10 mCi/mM), NaOAc-[2-$^{14}$C] (56 mCi/mM), L-methionine-[*methyl*-$^{14}$C] (59 mCi/mM) and dimethyl sulphate-[$^{14}$C] (20·6 mCi/mM) were obtained.

*Plant material and feeding techniques.* Bulbs were grown in pots in a greenhouse, and at the appropriate stage of growth, the plant was removed from the pot, soil washed from the roots, and the root ball was supported in a 250 ml beaker. Radioactive precursors were either dissolved in 10 ml $H_2O$ or administered as the sodium salts in phosphate buffer (0·1 M, pH 7·0). The soln was applied to the roots and when most had been taken up by the plant, additional $H_2O$ (*ca* 100 ml) was added. Further $H_2O$ was added as required during feeding period.

*Extraction of eucomin.* The top portion of the plant was discarded; bulb and root portion was sliced and homogenized in a blender with 300 ml 50% EtOH. The mixture was then poured into boiling EtOH (200 ml), the first extract decanted off, and the residue re-extracted with hot EtOH (2 × 200 ml). The combined filtered extracts were evaporated to about 40 ml, diluted with 100 ml $H_2O$ and extracted with $Et_2O$ (4 × 200 ml). The combined extracts were separated by TLC in solvent system A, and eucomin was eluted with MeOH–$Me_2CO$ (1:1). The eluted material was quantitated by its UV spectrum*; $\lambda_{max}$ 213 nm (log $\epsilon$ 4·48), 232 sh (4·16), 358 (4·49); and diluted with 15 mg inactive carrier. (Natural eucomin was employed since chemical synthesis [14] failed in our hands.) The diluted material was methylated in dry $Me_2CO$ using $Me_2SO_4$–$K_2CO_3$ and purified to constant sp. act. by TLC (solvent A) and repeated crystallization from aq. MeOH. The product was diluted again with synthetic dimethyleucomin prior to degradative studies.

*Synthesis of dimethyleucomin.* 2,4-Dimethoxy-6-hydroxyacetophenone [15] was converted into 5,7-dimethoxychromone using ethyl formate–Na [16] and hydrogenated over $PtO_2$ in HOAc to yield 5,7-dimethoxychroman-4-one. Dimethyleucomin was then prepared [17] by acid catalysed condensation of this chromanone with anisaldehyde in dry EtOH. The product was purified by TLC (solvent A) and recrystallized from aq. MeOH mp 141–2°, (lit. [1] 141–4°).

*Degradation of dimethyleucomin.* Dimethyleucomin (200 mg) was dissolved with warming in 20 ml *t*-BuOH and 200 mg $KClO_3$ in 10 ml $H_2O$ was added, followed by 1 ml 1% $OsO_4$. The mixture was left at room temp. overnight, then 50 ml *t*-BuOH was added, and the pptd salts removed by centrifugation. The supernatant was cooled in ice, 4 ml $H_2O_2$ (100 vol.) was added, followed dropwise by 4 ml 2 N NaOH and then a further 2 ml $H_2O_2$. The mixture was stirred for 1 hr, diluted with 50 ml $H_2O$ and extracted with $Et_2O$ (3 × 25 ml) to give the neutral extract. The residue was acidified (dil. HCl) and extracted with $Et_2O$ (3 × 25 ml) to yield the acidic extract. The neutral extract was evaporated to *ca* 50 ml to remove $Et_2O$, treated with a soln (25 ml) containing 1% $KMnO_4$ and 2% $Na_2CO_3$ over 0·75 hr with heating and stirring. After cooling and acidification, the mixture was decolorized with solid $Na_2S_2O_5$, and extracted with $Et_2O$ (3 × 25 ml). The residue after evapn was recrystallized from aq. MeOH and sublimed at 150°/0·09 mm to yield 73 mg anisic acid, mp and mmp 183–5°. Anisic acid was degraded further by micro Zeisel demethylation, collecting MeI in ethanolic $Et_3N$.

The acidic extract was evaporated and the black, osmium-containing residue taken up in a little $H_2O$ and treated with solid $Na_2S_2O_5$, after which it was re-extracted with $Et_2O$ (3 × 25 ml). After concn, the extracts were purified by TLC (solvent B), and 4,6-dimethoxysalicylic acid was eluted with MeOH, crystallized from aq. EtOH, and finally sublimed at 140°/0·1 mm. Yield 22 mg, mp and mmp 160–2°. 4,6-Dimethoxysalicylic acid was degraded further by Cu-quinoline decarboxylation, collecting $CO_2$ as $BaCO_3$. Alternatively, the acidic extract was evaporated to dryness and methylated by heating and stirring in 20 ml dry $Me_2CO$ with 0·3 ml $Me_2SO_4$ over 1 g $K_2CO_3$ for 1 hr. After filtering and evaporation, the product, 3,5,7-trimethoxychromone, was separated by TLC (solvent C), then hydrolysed without further purification by heating under reflux with 0·5 g KOH in 10 ml 50% EtOH for 5 hr. After acidification (dil. HCl) and EtOAc extraction (3 × 20 ml), the product was purified by TLC (solvent A) and recrystallization from aq. MeOH to yield 14 mg 2′-hydroxy-2,4′,6′-trimethoxyacetophenone mp and mmp 101–3°.

2-O-*Methylphloracetophenone*-[O-*methyl*-$^{14}$*C*]. 302 mg 4-*O*-benzoylphloracetophenone [18] (1·1 mM) was stirred under reflux in 12 ml dry $Me_2CO$ with 2 g $K_2CO_3$, 0·1 ml $Me_2SO_4$ and 1·5 mg $Me_2SO_4$-[$^{14}$C] (250 $\mu$Ci, total $Me_2SO_4$ 1·075 mM) for 1 hr. The mixture was worked up and the product treated with 25 ml 8% methanolic KOH at room temp. under $N_2$ for 2·5 hr. The reaction mixture was poured into 100 ml $H_2O$ and extracted with EtOAc (3 × 50 ml), the combined extracts were then washed with 50 ml 10% $NaHCO_3$, 25 ml $H_2O$, and evaporated. The product was purified by TLC (solvent A) and recrystallized from aq. MeOH to give 128 mg 2-*O*-methylphloracetophenone-[*O*-methyl-$^{14}$C] mp 207–10° (lit. [19] 205–7°).

2′,4′,4-*Trihydroxy*-6′-*methoxychalcone*-[*methyl*-$^{14}$*C*]. 36·8 mg 2-*O*-methylphloracetophenone-[*O*-*methyl*-$^{14}$C], 38·1 mg *p*-hydroxybenzaldehyde, 0·1 ml EtOH and 1 ml 100% w/v aq. KOH were heated in a closed flask at 100° for 0·5 hr, then left at room temp. for 48 hr. The mixture was cooled in ice, acidified with 50% HCl, diluted with $H_2O$ and extracted with EtOAc (3 × 25 ml). The product was separated by TLC (solvent D) and purified to constant sp. act. (2·05 × $10^8$ dpm/mM) by TLC (solvents C and E) to give 47 mg 2′,4′,4-trihydroxy-6′-methoxychalcone-[methyl-$^{14}$C] mp 261–3°† (recrystd. aq. MeOH) (lit. [20] 235°). $\lambda_{max}$ 370 nm (log $\epsilon$ 4·47).

2′,4′-*Dihydroxy*-4,6′-*dimethoxychalcone*-[6′-*methyl*-$^{14}$*C*]. 75·8 mg 2-*O*-methylphloracetophenone-[*O*-*methyl*-$^{14}$C]. 76 mg anisaldehyde and 1 ml EtOH were treated with aq. KOH as above. The product was isolated by TLC (solvent A) and purified by TLC (solvents C and E), recrystallization from aq. MeOH, and TLC (solvent E) to give 42 mg 2′,4′-dihydroxy-4,6′-dimethoxychalcone-[6′-*methyl*-$^{14}$*C*] mp 154–6°† (lit. [19] 169°). Sp. act. 2·04 × $10^8$ dpm/mM. $\lambda_{max}$ 368 nm (log $\epsilon$ 4·47).

* The considerably lower extinction coefficients reported by Tamm [1, 4] are unrealistic for a compound of this structure.

† Despite the discrepancies with published mps, both chalcones gave satisfactory microanalyses.

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