Proximity Effects in the Electron Impact Mass Spectra of Aurones and Related Compounds

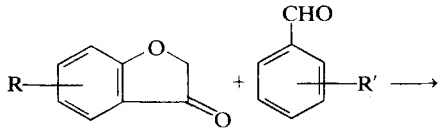
R. J. Goldsack
State Pollution Control Commission, Joseph Street, Lidcombe, NSW Australia 2141

J. S. Shannon
School of Chemistry, University of New South Wales, P.O. Box 1, Kensington, NSW Australia 2033

The principal ions in the electron impact mass spectra of a series of 6-methoxyaurones have been shown to be due to four separate reactions associated with proximity effects involving the phenyl group and the coumaran-one residue. A detailed study with labelled derivatives has been supplemented by a study of the vinylogue 2-cinnamylidene-6-methoxycoumaran-3-one and compounds in which the aurone phenyl group has been replaced by α-naphthyl, β-naphthyl and 9-anthryl.

INTRODUCTION

Early this century, a series of 2-benzylidenecoumaran-3-ones (**2**) were synthesized by von Auwers and his co-workers who condensed together a 1-benzofuran-3(2H)-one (**1**) and a benzaldehyde[1,2] (Scheme 1). Over



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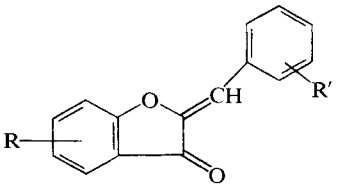

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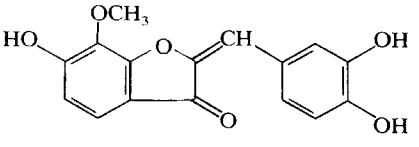
Scheme 1

30 years passed before representatives of this class of compound were found in nature. In 1943 Geissman *et al.*[3] discovered leptosidin (**3**) and its 6-glucoside in the ray florets of *Coreopsis grandiflora* Nutt., which belongs to the Asteraceae (daisies and their allies). Further work revealed that 2-benzylidenecoumaran-3-ones (**2**) occur mainly in flowers, petals and leaves of members of Coreopsidinae, a sub-tribe of the Asteraceae.[3] They are also found, however, in the corollas of the common snapdragon, *Antirrhinum majus* L. (Scrophulariaceae), the Bermuda buttercup, *Oxalis pes-caprae* L. (erroneously[4] called *O. cerna* Thunb. in Ref. 3), a member of the wood sorrel family (oxalidaceae), and in the monocotyledenous family Cyperaceae (sedges).



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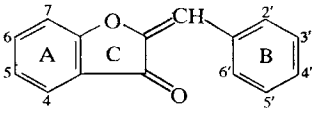

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For convenience Geissman and Bate-Smith[5] coined the generic term 'aurone' to describe compounds which are derivatives of 2-benzylidenecoumaran-3-one (**4**). The name, aurone, is analogous to flavone[3] and alludes to the beautiful yellow colours of some of the naturally occurring derivatives. Aurones are far less commonly encountered in nature than are flavones and isoflavones with which they are isomeric. Brady *et al.*[6] who have reported the electron impact (EI) mass spectra of some aurone epoxides have shown that it is possible for 6-methoxyaurones to exist as either *Z*- or *E*-isomers.

Although aurones are natural products the literature contains no references to their detailed mass spectra, whereas a significant number appears for the isomeric flavones and isoflavones. We have now studied a large series of 6-methoxyaurones (Table 1), together with labelled derivatives (Table 2). To supplement the study, we have synthesized the vinylogue 2-cinnamylidene-6-methoxycoumaran-3-one (**27**) and labelled derivatives.

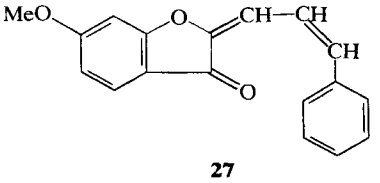

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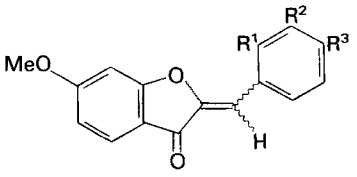
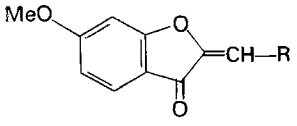
Table 1. Aurones studied[a,b]



	R^1	R^2	R^3	Stereochemistry
5	H	H	H	(*E*) and (*Z*)
6	NO_2	H	H	(*E*) and (*Z*)
7	H	NO_2	H	(*E*) and (*Z*)
8	H	H	NO_2	(*E*) and (*Z*)
9	CH_3O	H	H	(*E*)
10	H	CH_3O	H	(*E*)
11	H	H	CH_3O	(*E*)
12	CH_3	H	H	(*E*)
13	H	CH_3	H	(*E*)
14	H	H	CH_3	(*E*)
15	Cl	H	H	(*E*)
16	H	Cl	H	(*E*)
17	H	H	Cl	(*E*)
18	OH	H	H	(*E*)
19	H	OH	H	(*E*)
20	H	H	OH	(*E*)
21	NH_2	H	H	(*E*)
22	H	NH_2	H	(*E*)
23	H	H	NH_2	(*E*)



24	R = α-naphthyl	(*E*)
25	R = β-naphthyl	(*E*)
26	R = 9-anthryl	(*E*)

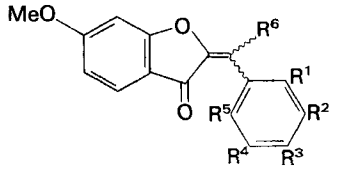
[a] The trivial name aurone expresses the molecule 2-benzylidene-1-benzofuran-3(2*H*)-one, with the geometry around the double bond unspecified. The systematic name of compound **9**, for instance, is (*E*)-6-methoxy-2-(2′-methoxy benzylidene)-1-benzofuran-3(2*H*)-one.

[b] For convenience, reference to the 6-methoxy group may be omitted when referring to 6-methoxyaurones possessing a substituent in ring B. For example, 6-methoxy-2′-nitroaurone may be called 2′-nitroaurone.

RESULTS AND DISCUSSION

At first sight the EI mass spectra of most 6-methoxyaurones (see Experimental and Table 4) are fairly simple. Nevertheless, they have interesting and distinguishing features associated *inter alia* with proximity effects. For a given substituent the spectra of 3′- and 4′-aurones are usually similar but different from their 2′-isomers due to the proximity effects, although these effects may also exist to some extent in some of the spectra of the 3′- and 4′-isomers. With the exception of the 2′-nitro- (**6**), 2′-methyl- (**12**), 2′-chloro- (**15**) and 2′-aminoaurones (**21**), the base peak is due to either the molecular ion or $[M-H]^+$ ion, and together these carry the major portion of the ion current. In no case was a difference found between the spectrum of a *Z*- and *E*-isomer either at 70 eV or at low energy.

Table 2. Labelled aurones studied



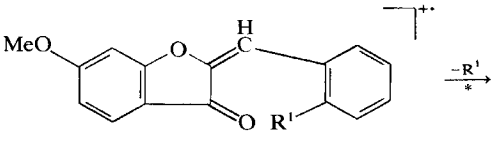
	R^1	R^2	R^3	R^4	R^5	R^6	Stereochemistry
5	H	H	H	H	H	H	(*E*) and (*Z*)
5a	D	D	D	D	D	H	(*E*)
5b	H	H	H	H	H	D	(*E*)
5c	D	H	D	H	D	H	(*E*)
5d	D	H	H	H	D	H	(*E*)
5e	H	D	H	D	H	H	(*E*)
5f	H	H	D	H	H	H	(*E*)
8a	D	H	NO_2	H	D	H	(*E*)
9a	CH_3O	D	H	D	H	H	(*E*)
9b	D	H	CH_3O	H	D	H	(*E*)
11a	D	H	CH_3O	H	D	H	(*E*)
13a	H	CD_3	H	H	H	D	(*E*)
13b	H	H	CD_3	H	H	H	(*E*)
13c	D	H	CH_3	H	D	H	(*E*)

Presumably, the double bond around which the isomerism is manifest in the neutral molecule loses much of its double bond character following ionization.

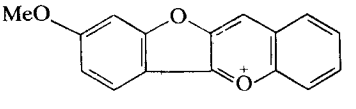
Proximity effects

The formation of many of the main ions in the spectra are due to proximity effects involving a 2′-substituent (R^1) and ring C. These effects lead to at least four separate reactions. One is the not unexpected loss of the 2′-substituent, while the other three occur when the 2′-substituent contains hydrogen (R^1 = OH, NH_2, CH_3) and the first step in each of these latter reactions is the transfer of a hydrogen atom from R^1 (confirmed by deuterium labelling) to sites in ring C. Each of the four reactions will be discussed separately, as will a related case where $R^1 = NO_2$.

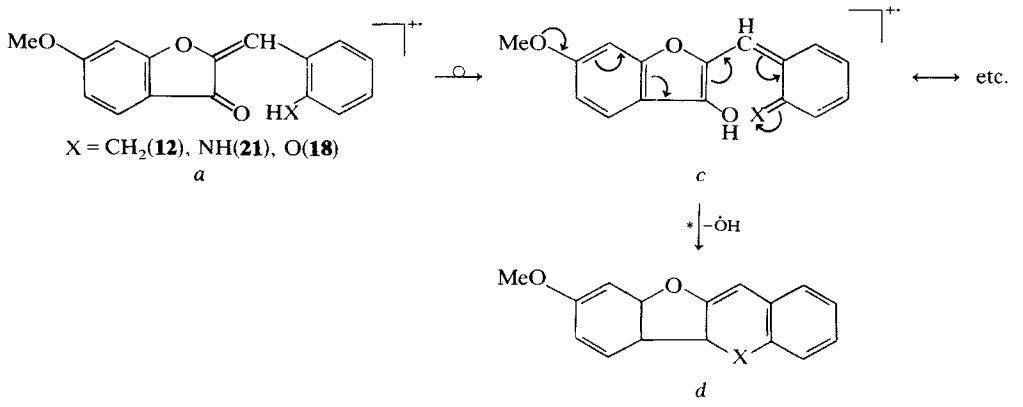
(1) When R^1 = H, CH_3, Cl, OCH_3 an abundant $[M-R^1]^+$ ion is formed at *m/z* 251. Study of deuterated derivatives **5a** and **5b** confirmed that the hydrogen atom lost from the molecular ion of **5** is from ring B, and thus the $[M-R^1]^+$ ion may have the stable oxonium structure *b*. Examples of this type of reaction in other systems where ring closure is assumed to occur to form stable oxonium ions have been reported previously.[7]



a



b

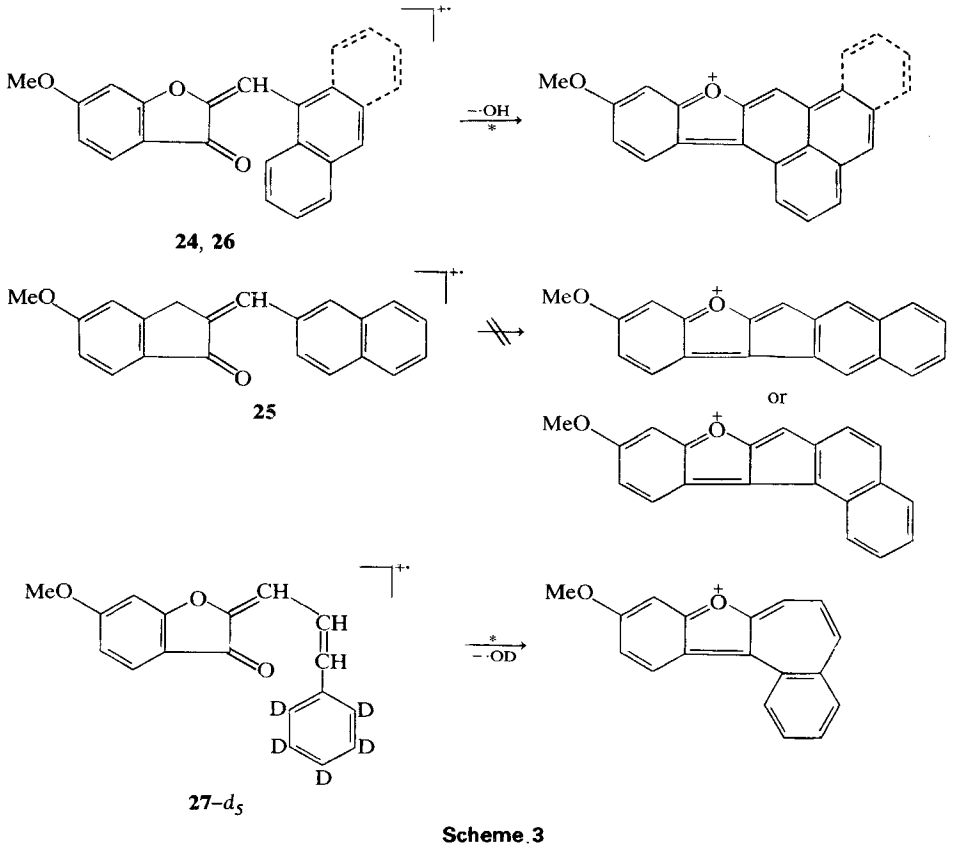


Scheme 2

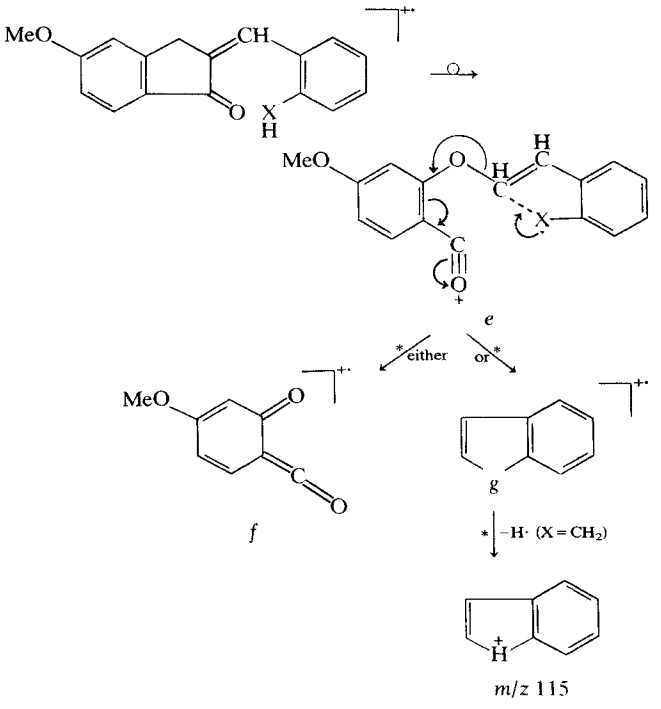
(2) When $R^1 = OH$, NH_2, CH_3 there are $[M-OH]^+$ ions in each spectrum and metastable evidence showed that these were formed from the molecular ions. Thus, two different proximity effects operate with 2′-methylaurone (**12**) whereby both $[M-15]^+$ and $[M-17]^+$ ions appear. Further, when $R^1 = OH$ labelling of the carbonyl oxygen atom confirmed that it was this atom which was lost as OH and not the oxygen atom in R^1 as may have been presumed by analogy with the results when $R^1 = OMe$, above. Deuterium labelling of R^1 in the three compounds (**18**, **21**, **12**) also confirmed that the hydrogen lost in the OH group originated from the 2′-substituent (R^1). The reaction can thus be formulated as shown in Scheme 2. The driving force for the initial hydrogen transfer is presumably due to the rearranged molecular ion *c* having greater resonance stabilization than *a*. In *c*, for example, there is extended conjugation from the 6-methoxy group through to the 2′-substituent—similar conjugation is absent in *a*.

There is also loss of OH both in the ion source and field free region from the molecular ions of **24**, **26** and **27**, but not from **25**. In these cases the driving force is not simply associated with the favourable proximity of the carbonyl group but with the stability of the $[M-17]^+$ ions. With **24**, the hydrogen lost in OH is transferred via an 8-membered system and with **27** through a 9-membered system (confirmed by deuterium labelling). If the molecular ion of **25** reacted similarly, the necessary hydrogen transfer would be through a 7-membered system, but the consequent $[M-17]^+$ ion would contain a less favourable system of two fused 5-membered rings. Presumably, for similar reasons the simple aurones where ring B is benzenoid do not give equivalent $[M-17]^+$ ions (Scheme 3).

(3) When the 2′-substituent (R^1) is NH_2, OH, CH_3



Scheme 3



Scheme 4

(or NO_2), the coumaran residue is split off and the charge may remain on either of the two fragments depending upon the relative ionization energies of the corresponding neutral molecules. Since this reaction occurs only when R^1 contains hydrogen (except when $R^1 = NO_2$) a hydrogen transfer step is apparently involved, (oxygen transfer when $R = NO_2$, see below) leading to a rearranged molecular ion *e* which may cleave to yield stable fragments (Scheme 4).

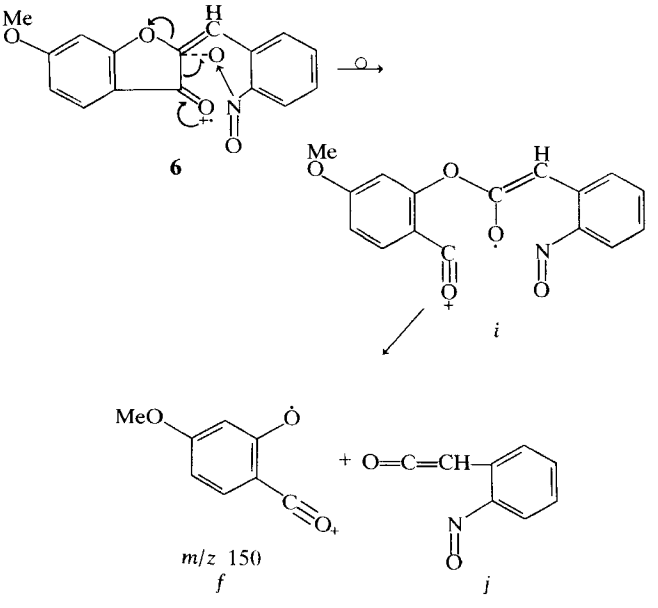
The observed relative abundances of the ions *f* and *g* may be explained in terms of the ionization energy values of the neutral molecules corresponding to *g*. For example (Table 3), charge retention is favoured by the fragment of low ionization energy. Although no ionization energy value is available for the molecule corresponding to *f* it is apparently less than 8.3 eV, thus accounting for the observation that ion *f* produces the base peak when X = oxygen.

When $X = CH_2$, ion *g* loses a hydrogen atom to produce an ion at *m/z* 115, and thus the abundances of the ions at *m/z* 116 and 115 have been summed for comparison with the abundances of ion *g* when X = O and NH. The loss of a hydrogen atom from ion *g* when $X = CH_2$ but not when X = O, NH is expected from comparison with the spectra of indene, benzofuran and indole, a strong $[M-1]^+$ ion (84.5%) being observed only with indene.

The spectrum of the 2′-nitroaurone (**6**) is quite different from its *meta* (**7**) and *para* (**8**) isomers. For example, it lacks $[M]^{+\cdot}$, $[M-H]^+$ and $[M-NO_2]^+$ ions but has a unique base peak at *m/z* 150 due, presumably, to a proximity effect. The formation of this ion at *m/z* 150 may be analogous to the formation of ion *f* above where $R^1 = CH_3$, NH_2, OH. In this case, however, an oxygen and not a hydrogen atom is transferred via a 6-membered system and the less sterically favoured 5-membered system ruptured to yield the intermediate ion *i* or the fragments *f* and *j* in one step (Scheme 5).

There is no ion corresponding to the neutral product as in the previous cases, since here the fragment *j* possesses a deactivating electron-attracting group.

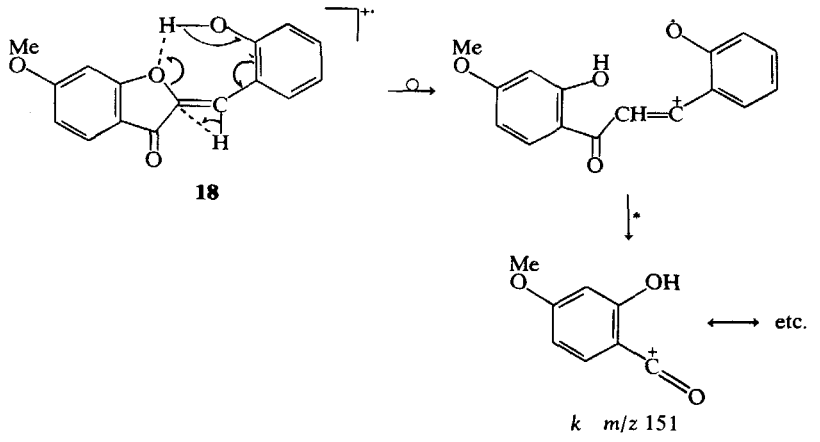
(4) So far we have dealt with two reactions, one of which is triggered by hydrogen transfer from the 2′-substituent to carbonyl oxygen [(2) above] and the other by hydrogen transfer to C-2 [(3) above]. Proximity effects lead to yet a third reaction both in the ion source and field free region, involving hydrogen transfer from the 2′-substituent (NH_2, OH, CH_3) (confirmed by deuterium labelling), this time to form an ion at *m/z* 151 (Scheme 6). A likely receptor for the hydrogen atom appears to be the ether–oxygen atom whereby a stable ion *k* is produced, the molecular formula of which was confirmed by accurate mass measurement.



Scheme 5

Table 3. Relative abundances of *f* and *g* fragments and ionization energies of neutral *g* fragments

X	*f*		*g*		Ionization energy[a]
NH	*m/z* 150	0%	*m/z* 117	100%	7.78 eV
CH_2	*m/z* 150	4%	*m/z* (116+115)	36%	8.13 eV
O	*m/z* 150	100%	*m/z* 118	50%	8.3 eV



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k m/z 151

EI mass spectra of 3'- and 4'-substituted aurones

In several examples, the reactions (1) and (2) ascribed above to proximity effects appear to persist in the 3'-substituted derivatives and even in the 4'-substituted compounds. For example, when R^2 and $R^3 = CH_3$ although the $[M-1]^+$ ion (*b*) is as expected the most abundant reflecting the presence of unsubstituted 2'-positions, nevertheless, there are other abundant ions at $[M-15]^+$ due to the loss of the 3'- or 4'-methyl group as confirmed by study of the labelled derivatives **13a** and **13b**. The 3'- and 4'-OMe derivatives similarly give $[M-OMe]^+$ ions, although there is a marked diminution in abundance as the position of the OMe group changes from 2'- to 3'- to 4'-. A similar situation is observed when R^1, R^2 and $R^3 = Cl$. In the spectra of 3'-NH_2 and 3'-OH compounds but not in the spectrum of the 3'-CH_3 derivative there were $[M-OH]^+$ ions seemingly analogous to those encountered in the 2'-derivatives. Further work is in progress to resolve the relationship of these reactions of the 2'-, 3'- and 4'-substituted derivatives.

Reactions involving consecutive loss of free radicals

The EI mass spectra of the aurones are marked by examples where even-electron ions eject a free radical to form an odd-electron ion, an exceptional phenomenon in organic mass spectrometry. For example, the $[M-R^1]^+$ ions generally lose a methyl radical to produce an ion at $[M-(R^1+Me)]^{+\cdot}$—deuterium labelling showed that the methyl group originated from the 6-methoxy group (Scheme 7).

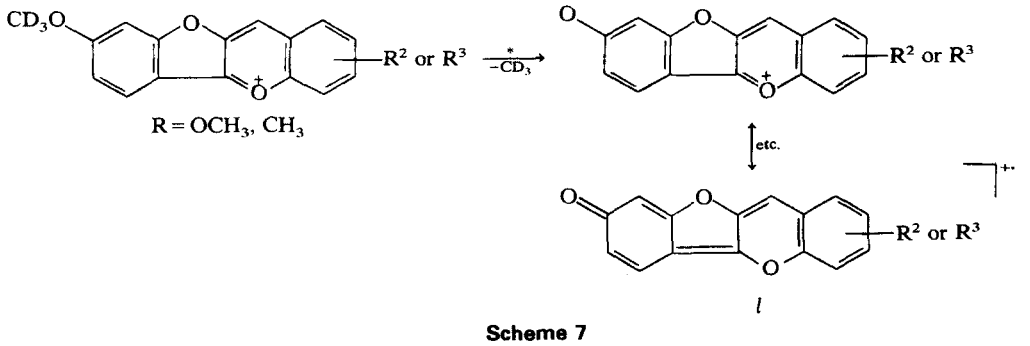
The stability of *l* may be due to the occurrence of several important resonance forms in which the charge can reside on any of the three oxygen atoms.

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were recorded using either a Varian Associates A60 NMR spectrometer or a HA 100 NMR spectrometer. Except where stated, deuteriochloroform was the solvent. Chemical shifts are quoted in ppm relative to tetramethylsilane. Mass spectra were determined on an AEI MS 12 mass spectrometer: ionizing energy, 70 eV; trap current, 100 μA; accelerating voltage, 8 kV; source temperature, *c.* 175 °C. Isotopic analyses were determined using the same conditions except that the trap current was reduced to 20 μA, and the ionization voltage was reduced below the appearance energy of the $[M-H]^+$ ion. All mass spectra were recorded in the scan decrease mode. High resolution mass spectra were determined on an AEI MS 9 mass spectrometer at the CSIRO Division of Entomology, Canberra. Microanalyses were performed either by Dr Challen and Mr J. Sussman of the University of New South Wales or the Australian Microanalytical Service.

6-Methoxy coumaran-3-one was prepared using the method of von Auwers and Pohl.[2]

6-Methoxycoumaran-3-one-carbonyl-^{18}O. A mixture of 6-methoxycoumaran-3-one (10 mg), dry dioxan



R = OCH_3, CH_3

l

Scheme 7

(150 μl), $H_2{}^{18}O$ (5.0 μl; Miles Laboratories, Inc., 96.56 atom % ^{18}O) and trifluoroacetic acid (1.0 μl) was heated in a Pierce Reacti-Vial at 110 °C for 72 h. The solvent was removed by freeze drying and the residue used without further purification. Low voltage mass spectrometry showed that an incorporation of 45.8% of ^{18}O had been achieved.

(*E*)-6-Methoxyaurone-2′,4′,6′-d_3. *m*-Toluidine-2,4,6-d_3 (d_1, 0.6; d_2, 2.9, d_3, 96.5%) was prepared in a manner similar to that described for the preparation of aniline -2,4,6-d_3.[9] The labelled amine salt was converted[9] to toluene -2,4,6-d_3, which was oxidized to benzaldehyde -2,4,6-d_3 with chromyl chloride.[10] The title compound (540 mg, m.p. 145–146 °C) was prepared from benzaldehyde -2,4,6-d_3 (220 mg) and 6-methoxycoumaran-3-one (450 mg) in a similar manner to that given above for the unlabelled analogue. Isotopic analysis: d_2, 2.4; d_3, 97.2; d_4, 0.3%. The NMR spectrum did not show a signal at δ 7.90 due to the 2′- and 6′-protons,[6] but a singlet at δ 7.42 due to the 3′- and 5′-protons was present.

(*E*)-6-Methoxyaurone-2′,3′,4′,5′,6′-d_5. Benzaldehyde-2,3,4,5,6-d_5 was prepared from benzene-d_6 using an aldehyde synthesis devised by Rieche *et al.*[11] 6-Methoxycoumaran-3-one (200 mg) and benzaldehyde-d_5 (200 mg) were dissolved in absolute ethanol (1.0 cm^3) and piperidine (1 drop) was added. After standing at ambient temperature for 15 h, the crude product was collected on a filter and washed with a small quantity of ice-cold ethanol. Pure title compound (75 mg), m.p. 146–148 °C, was obtained after elution from silica gel (5.0 g) with benzene and crystallization from ethanol. Isotopic analysis: d_5, 100%. The NMR spectrum showed no signals that could be attributed to the 2′-, 3′-, 4′-, 5′- and 6′-protons.

(*E*)-6-Methoxyaurone-2′,6′-d_2. Crude 4′-amino-6-methoxyaurone-2′,6′-d_2 (30 mg) (see below) was deaminated using the method of Renaud *et al.*[12] Purification was effected by a chromatography on silica gel using benzene as the eluant. The title compound (8.4 mg) melted at 145–147 °C after recrystallization from ethanol. The yield was low due to spillage. Isotopic analysis: d_2, 100%.

(*E*)-6-Methoxyaurone-3′,5′-d_2. 4-Bromoaniline-2,6-d_2 deuteriochloride was prepared by the repeated exchange of 4-bromoaniline hydrochloride with D_2O until the NMR spectrum showed the absence of protium *ortho* to the amino group. Isotopic analysis: d_1, 1.8; d_2, 92.6; d_3, 5.6%. The amine salt was converted to the corresponding diazonium salt which was reduced to bromobenzene-3,5-d_2 with alkaline formaldehyde.[9] Treatment of the labelled Grignard reagent[13] with ethoxymethyleneaniline[14] gave benzaldehyde-3,5-d_2, which was converted to *trans*-6-methoxyaurone-3′,5′-d_2 using the procedure given for the preparation of the 2′,4′-6′-d_3 analogue. The title compound crystallized from methanol as pale yellow needles, m.p. 148–150 °C. Isotopic analysis: d_1, 1.1; d_2, 97.8; d_3, 1.1%.

(*E*)-6-Methoxyaurone-4′-*d*. Toluene-4-*d* prepared by quenching 4-tolylmagnesium bromide with D_2O,[13] was converted to benzyl bromide -4-*d* with *N*-bromosuccimide. The benzyl halide was oxidized to benzaldehyde-4-*d* with hexamethylenetetramine.[15] The title compound was prepared by condensing 6-methoxycoumaran-3-one and benzaldehyde using the method given for the preparation of the 2′,4′,6′-d_3 compound. It crystallized from methanol as pale yellow needles, m.p. 147–149 °C. Isotopic analysis: d_0, 12.4; d_1, 87.6%.

(*E*)-6-Methoxyaurone-α-*d*. Benzaldehyde-α-*d* was prepared using the method of Staab and Braunling[14] except that lithium aluminium deuteride was used instead of lithium aluminium hydride. The title compound m.p. 146–148 °C was prepared using a procedure similar to that described for the unlabelled compound. The NMR spectrum showed no signal at δ 6.8 due to the α-proton.[6] Isotopic analysis: d_1, 100%.

(*E*)-6-Methoxyaurone-carbonyl-^{18}O. A solution of (*E*)-6-methoxyaurone (5 mg) in dry dioxane (100 μl) containing $H_2{}^{18}O$ (5.0 μl; Miles Laboratories, Inc., 96.56 atom % ^{18}O) and trifluoroacetic acid (1.0 μl) was heated at 110 °C for 48 h in a Pierce Reacti-Vial. The volatile materials were removed by freeze drying and the residue used without further purification. Isotopic analysis showed the ^{18}O content to be 40.8%.

(*Z*)-6-Methoxyaurone. A solution of (*E*)-6-methoxyaurone (200 mg) in sodium dried benzene (20 cm^3) was irradiated for 2 h. Removal of the solvent afforded a mixture of (*E*)- and (*Z*)-isomers. Repeated preparative thin-layer chromatography (TLC) gave (*E*)-6-methoxyaurone (45 mg) which crystallized from methanol as pale yellow needles, m.p. 137–139 °C (lit.[6] m.p. 136–137 °C). The NMR spectrum was similar to that reported previously.[6]

(*E*)-6-Methoxy-2′-nitroaurone. The following procedure is a general aurone synthesis devised by Geissman and Harborne;[16] it is superior to that of von Auwers and Pohl[2] because the yields are greater and the product is not contaminated with dark-coloured products that are difficult to remove by crystallization techniques.

Concentrated hydrochloric acid (0.8 cm^3) was added to a solution of 6-methoxycoumaran-3-one (765 mg) and 2-nitrobenzaldehyde (604 mg) in glacial acetic acid (20 cm^3). After standing at ambient temperature for 16 h, the crude product was collected on a filter and washed with a small quantity of glacial acetic acid. Recrystallization from glacial acetic acid afforded pure title compound (980 mg) m.p. 201–203 °C.

(*Z*)-6-Methoxy-2′-nitroaurone was prepared from the corresponding (*E*)-isomer (50 mg) in a similar way to that described for the preparation of (*Z*)-6-methoxyaurone. The title compound (22 mg) crystallized from ethanol as pale yellow needles, m.p. 203–206 °C. (Found: C, 64.5; H, 3.5; N, 4.5. $C_{16}H_{11}NO_5$ requires C, 64.6; H, 3.7; N, 4.7%).

(*E*)-6-Methoxy-3′-nitroaurone was prepared from 6-methoxycoumaran-3-one (328 mg) and 3-nitrobenzaldehyde (300 mg) using the procedure described for the preparation of the corresponding 2′-nitro compound. The pure product crystallized from glacial acetic acid as very pale yellow needles, m.p. 213–214 °C. (Found: C, 64.6; H, 3.7; N, 4.5. $C_{16}H_{11}NO_5$ requires C, 64.6; H, 3.7; N, 4.7%).

(*Z*)-6-Methoxy-3′-nitroaurone was prepared from the corresponding (*E*)-isomer (93 mg) using the method described for the preparation of (*Z*)-6-methoxyaurone. Pure (*Z*)-6-methoxy-3′-nitroaurone (19 mg) crystallized from glacial acetic acid as long yellow needles, m.p. 182–184 °C (Found: C, 64.5; H, 3.9; N, 4.8. $C_{16}H_{11}NO_5$ requires C, 64.6; H, 3.7; N, 4.7%).

(*E*)-6-Methoxy-4′-nitroaurone was prepared from 6-methoxycoumaran-3-one (328 mg) and 4-nitrobenzaldehyde (300 mg) using the method described for the synthesis of the (*E*)-2′-nitro compound. The title compound (460 mg) crystallized from glacial acetic acid as red needles, m.p. 255–258 °C (Found: C, 64.5; H, 3.8; N, 4.8. $C_{16}H_{11}NO_5$ requires C, 64.6; H, 3.7; N, 4.7%).

(*E*)-6-Methoxy-4′-nitroaurone-2′,6′-d_2. 4-Nitroaniline-2,6-d_2 hydrochloride was prepared in a similar manner to aniline-2,4,6-d_3.[9] Isotopic analysis: d_2, 100%. The 4-nitroaniline-2,6-d_2 hydrochloride was converted to 4-nitrobenzaldehyde-2,6-d_2 by the method of Beech.[17]

The title compound was prepared from the labelled aldehyde and 6-methoxycoumaran-3-one using the method given for the unlabelled analogue.

(*Z*)-6-Methoxy-4′-nitroaurone. The (*E*)-isomer (100 mg) was dissolved in chloroform (25 cm^3) and irradiated by standing in bright Sydney sunshine for 6 days. The solvent was removed and the residue gave (*E*)-6-methoxy-4′-nitroaurone after purification by preparative TLC (benzene) and recrystallization from benzene. The title compound (18 mg) was obtained as pale yellow needles, m.p. 224–227 °C. (Found: C, 64.9; H, 3.8; N, 4.7. $C_{16}H_{11}NO_5$ requires C, 64.6; H, 3.7; N, 4.7%).

(*E*)-2′,6-Dimethoxyaurone was prepared from 6-methoxycoumaran-3-one (380 mg) and 2-methoxybenzaldehyde (600 mg) using the method described for the preparation of (*E*)-6-methoxy-2′-nitroaurone. The title compound (520 mg) crystallized from ethanol as pale yellow needles, m.p. 156–158 °C. (Found: C, 72.6; H, 5.1. $C_{17}H_{14}O_4$ requires C, 72.3; H, 5.0%).

(*E*)-2′,6-Dimethoxyaurone-3,5-d_2. 2-Methylphenol-4,6-d_2 was prepared using the method of Bothner-By and Moser[18] and then the methyl ether was oxidized to 2-methoxybenzaldehyde-3,5-d_2 by the Sommelet reaction[15] via the benzyl bromide. The labelled aldehyde (102 mg) and 6-methoxycoumaran-3-one (102 mg) were dissolved in absolute ethanol (3 cm^3) with the aid of gentle heating and piperidine (1 drop) was added. After standing at room temperature for 1 h, the crude product was collected on a filter and wahsed with a little ice-cold ethanol and recrystallized from ethanol. Pure (*E*)-2′,6′-dimethoxyaurone-3,5-d_2 (99 mg) was obtained as pale yellow needles, m.p. 159–161 °C. Isotopic analysis: d_1, 6.4; d_2, 79.9; d_3 11.1; d_4, 2.5%.

2′-(*E*)-Trideuteromethoxy-6-methoxyaurone. A solution of (*E*)-6-methoxy-2′-hydroxyaurone (67 mg) (see below) and trideuteromethyl iodide (0.10 cm^3) in dry (potassium carbonate) acetone (1.5 cm^3) was heated under reflux for 1.5 h with anhydrous potassium carbonate (250 mg). After the removal of the inorganic salts and volatile materials, the residue was dissolved in chloroform (10 cm^3) and washed successively with N sodium hydroxide (1 cm^3) and water. Removal of the solvent gave pure title compound in almost quantitative yield. Isotopic analysis d_3, 100.0%.

(*E*)-3′,6-Dimethoxyaurone was prepared from 3-methoxybenzaldehyde and 6-methoxycoumaran-3-one using the same procedure described for the preparation of the (*E*)-2′-methoxy compound. Pure (*E*)-3′,6-dimethoxyaurone (510 mg) crystallized from ethanol as yellow needles, m.p. 125–127 °C. (Found: C, 72.4; H, 5.1. $C_{17}H_{14}O_4$ requires C, 72.3; H, 5.0%).

(*E*)-3′-Trideuterio-6-methoxyaurone was prepared from (*E*)-3′-hydroxy-6-methoxyaurone (see below) using the method described for the preparation of the (*E*)-2′-trideuteriomethoxy isomer.

(*E*)-4′,6-Dimethoxyaurone was prepared from 4-methoxybenzaldehyde and 6-methoxycoumaran-3-one using the method given above for the preparation of the (*Z*)-2′-methoxy compound. The pure product crystallized from ethanol as yellow needles, m.p. 134–136 °C (lit.[19] m.p. 133°).

(*E*)-4′,6-Dimethoxyaurone-2′,6′-d_2. 4-Methoxyaniline deuteriochloride-2,6-d_2 was prepared in a similar manner to aniline-2,4,6-d_3.[9] Using the procedure described previously for the preparation of 4-nitrobenzaldehyde-2,6-d_2, the amine salt was converted to 4-methoxybenzaldehyde-2,6-d_2. The title compound was prepared by condensing together 6-methoxycoumaran-3-one (25 mg) and 4-methoxybenzaldehyde-2,6-d_2 (18 mg) dissolved in ethanol (0.25 cm^3) containing a trace of piperidine. The solvent was removed and the residue gave (*E*)-4′,6′-dimethoxyaurone-2,6-d_2 (30 mg) after chromatography on silica gel (25 mg) using benzene as the eluting solvent. Isotopic analysis: d_1, 2.2; d_2, 95.4; d_3, 2.4%.

(*E*)-4′-Trideuteriomethoxy-6-methoxyaurone was prepared from the corresponding hydroxy compound using the procedure described for the preparation of the 2′-trideuteriomethoxy compound.

(*E*)-6-Methoxy-2′-methylaurone was prepared by allowing a solution of 6-methoxycoumaran-3-one (300 mg) and 2-methylbenzaldehyde in absolute ethanol (4 cm^3) containing piperidine (3 drops) to stand at room temperature for *c.* 24 h. The orange-red

material that separated was collected on a filter and washed with a small quantity of ethanol. Recrystallization of this material gave pure (*E*)-6-methoxy-2′-methylaurone as very pale pink needles, m.p. 141–143 °C (Found: C, 76.9; H, 5.4, $C_{17}H_{14}O_3$ requires C, 76.7; H, 5.3%).

(*E*)-6-Methoxy-2′-trideuteriomethylaurone. Toluene-α-d_3 (isotopic analysis: d_3, 100%) was prepared by the method of Valade *et al.*[20] which was modified in that excess phenyl lithium was converted to benzoic acid by bubbling *dry* carbon dioxide through the reaction mixture. A mixture of 2- and 4-trideuteriomethyl benzaldehyde was prepared from toluene-α-d_3 using an aromatic aldehyde synthesis devised by Rieche *et al.*[11] The aldehydes were separated by preparative gas-liquid chromatography using a 5.5 m × 9.5 mm column packed with 30% Carbowax 30M on acid washed DCMS treated Chromosorb W (60–80 mesh). The temperature was 175 °C and the nitrogen flow rate was 150 ml min^{-1}. 2-Trideuteriomethylbenzaldehyde was converted to the title compound using the procedure given for the preparation of the unlabelled analogue. The pure product crystallized from ethanol as small pink needles, m.p. 141–143 °C. Isotopic analysis: d_2, 2.9; d_3, 97.1%.

(*E*) and (*Z*)-6-Methoxy-3′-methylaurone was prepared from 3-methylbenzaldehyde and 6-methoxycoumaran-3-one using the method described for the preparation of the 2′-methyl isomer. After recrystallization from ethanol, the title compound was obtained as colourless needles, m.p. 110–140 °C. Analysis by TLC showed that the product was a mixture of (*Z*)- and (*E*)-isomers. (Found: C, 76.8; H, 5.4. $C_{17}H_{14}O_3$ requires C, 76.7; H, 5.3%).

(*E*)-6-Methoxy-3′-trideuteriomethylaurone-α-*d*. *m*-Xylene-d_6 was prepared using the method of Chen *et al.*[21] This was oxidized, via the benzyl halide, to 3-trideuteriomethylbenzaldehyde-α-*d* by the Sommolet reaction.[15] The labelled aldehyde was converted to the title compound using the method given for the synthesis of the unlabelled analogue. Isotopic analysis: d_2, 2.7; d_3, 19.8; d_4, 77.5%.

(*E*)-6-Methoxy-4′-methylaurone. A solution of 6-methoxycoumaran-3-one (380 mg) and 4-methylbenzaldehyde (600 mg) in glacial acetic acid (10 cm^3) containing 10 N hydrochloric acid (0.4 cm^3) was allowed to stand at room temperature for 16 h. As no product separated during this period, the whole was poured into water (40 cm^3), and, after standing for 0.5 h, the precipitate was collected on a filter. Recrystallization of this material from ethanol afforded pure (*E*)-6-methoxy-4′-methylaurone as pale yellow needles, m.p. 155–157 °C. (Found: C, 76.6; H, 5.2. $C_{17}H_{14}O_3$ requires C, 76.7; H, 5.3%).

(*E*)-6-Methoxy-4′-methylaurone-2′,6′-d_2. 4-Methylaniline-2,6-d_2 hydrochloride which had been made by repeatedly exchanging 4-methylaniline hydrochloride with deuterium oxide, was converted to 4-methylbenzaldehyde-2,6-d_2 using the procedure given above for the preparation of 4-nitrobenzaldehyde-2,6-d_2. The title compound was prepared from 4-methylbenzaldehyde-2,6-d_2 and 6-methoxycoumaran-3-one using the method described for the preparation of (*E*)-6-methoxycoumaran-3-one using the method described for the preparation of (*E*)-6-methoxy-2′-methylaurone. After recrystallization from ethanol, the pure product was obtained as pale yellow needles, m.p. 155–158 °C. Isotopic analysis: d_1, 2.2; d_2, 97.4; d_3, 0.4%.

(*E*)-6-Methoxy-4′-trideuteriomethylaurone was prepared from 6-methoxycoumaran-3-one and 4-trideuteriomethylbenzaldehyde (see above) using the methods described for the preparation of (*E*)-6-methoxy-2′-trideuteriomethylaurone. Pure title compound was obtained as pale pink needles, m.p. 156–159 °C. Isotopic analysis: d_2, 2.9; d_3, 97.1%.

(*E*)-2′-Chloro-6-methoxyaurone was prepared from 6-methoxycoumaran-3-one (300 mg) and 2-chlorobenzaldehyde (300 mg) using the method described for the preparation of (*E*)-6-methoxy-2′-methylaurone. Pure title compound crystallized from ethanol as colourless needles (103 mg), m.p. 165–167 °C. (Found: C, 67.3; H, 3.9. $C_{16}H_{11}O_3Cl$ requires C, 67.0; H, 3.9%).

(*E*)-3′-Chloro-6-methoxyaurone was prepared from 6-methoxycoumaran-3-one (300 mg) and 3-chlorobenzaldehyde (300 mg) using the procedure described for the preparation of (*E*)-6-methoxy-2′-nitroaurone. Pure title compound (250 mg) crystallized from galcial acetic acid as pale orange needles, m.p. 148–150 °C. (Found: C, 67.1; H, 3.7. $C_{16}H_{13}O_3Cl$ requires C, 67.0; H, 3.9%).

(*E*)-4′-Chloro-6-methoxyaurone. A solution of 6-methoxycoumaran-3-one (300 mg) and 4-chlorobenzaldehyde (300 mg) in ethanol (4.0 cm^3) containing 12.5 M sodium hydroxide (0.1 cm^3) was allowed to stand at ambient temperature for 48 h and then heated on a boiling water bath for 0.5 h. After cooling in ice, the product was collected on a filter, washed with 70% v/v aqueous ethanol and recrystallized from glacial acetic acid. Pure (*E*)-4′-chloro-6-methoxyaurone (260 mg) was obtained as fine colourless needles, m.p. 170–171 °C. (Found: C, 67.15; H, 4.0. $C_{16}H_{11}O_3Cl$ requires C, 67.0; H, 3.9%).

(*E*)-2′-Hydroxy-6-methoxyaurone was prepared according to the method of Desai and Ray.[22] The product melted at 266 °C (dec.), (lit.[22] m.p. 258–259 °C).

(*E*)-2′-Hydroxy-6-methoxyaurone-carbonyl-^{18}O was prepared from 6-methoxycoumaran-3-one-carbonyl-^{18}O, (see above) and salicylaldehyde using the procedure of Desai and Ray.[22] Low voltage mass spectrometry showed that an incorporation of 45.8% of ^{18}O had been achieved.

(*E*)-3′-Hydroxy-6-methoxycoumaran-3-one was prepared from 6-methoxycoumaran-3-one (400 mg) and

3-hydroxybenzaldehyde (600 mg) using the method described for the preparation of (*E*)-6-methoxy-2′-nitroaurone. Pure title compound (415 mg) crystallized from ethyl acetate as golden flakes, m.p. 219–220 °C. (Found: C, 71.5; H, 4.5. $C_{16}H_{12}O_4$ requires C, 71.6; H, 4.5%).

(*E*)-4′-Hydroxy-6-methoxyaurone was prepared from 6-methoxycoumaran-3-one (400 mg) and 4-hydroxybenzaldehyde (600 mg) using the method given for the preparation of (*E*)-6-methoxy-2′-nitroaurone. Pure title compound (370 mg) crystallized from glacial acetic acid as very tiny orange-brown needles, m.p. 216–219 °C. (lit.[23] m.p. 210–211 °C).

(*E*)-2′-Amino-6-methoxyaurone. A solution of stannous chloride dihydrate (700 mg) in 10 N hydrochloric acid (3.0 cm^3) was added dropwise to a stirred solution of (*E*)-6-methoxy-2′-nitroaurone (150 mg) in hot (*c.* 60 °C) glacial acetic acid (10 cm^3). At the completion of the addition, the reaction mixture was heated on a boiling water bath for 0.75 h, cooled to *c.* 10° C and poured into 17 M sodium hydroxide solution. The precipitate was collected on a filter, washed thoroughly with water and chromatographed on silica gel (40 g) using chloroform as the eluting solvent. Pure title compound (41 mg) crystallized from benzene as red needles, m.p. 213–216 °C (lit.[6] m.p. 215 °C).

(*E*)-3′-Amino-6-methoxyaurone was prepared from the corresponding nitro compound using the procedure described for the preparation of the 2′-amino compound. The title compound was obtained as clusters of bright yellow needles (benzene), m.p. 160–161 °C. (Found: C, 71.8; H, 4.9; N, 5.4. $C_{16}H_{13}NO_3$ requires C, 71.5; H, 4.9; N, 5.2%).

(*E*)-4′-Amino-6-methoxyaurone was prepared from the corresponding nitro compound using the method described for the preparation of the 2′-amino derivative. Pure (*E*)-4′-amino-6-methoxyaurone crystallized from methanol–chloroform as fine bright yellow needles, m.p. 213–216 °C. (Found: C, 71.7; H, 4.85; N, 5.5. $C_{16}H_{13}NO_3$ requires C, 71.5; H, 4.9; H, 5.2%).

(*E*)-(*b′*)-Benzo-6-methoxyaurone was prepared from 1-naphthaldehyde and 6-methoxycoumaran-3-one using the previously described method for the preparation of (*E*)-6-methoxyaurone. Purification was effected by recrystallization from ethanol containing a trace of sodium hydroxide. Pure title compound was obtained as pale yellow needles, m.p. 176–177 °C. (Found: C, 79.55; H, 4.7. $C_{20}H_{14}O_3$ requires C, 79.95; H, 4.7%).

(*E*)-6-Methoxy-2-(1-naphthylidene-2*d*)-coumaran-3-one. 2-Methoxy-1-methylnaphthalene was demethylated[17] to give 2-hydroxy-1-methylnaphthalene, which was converted to 2-amino-1-methylnaphthalene by the Buchere reaction.[24] Diazotization of the amine followed by reduction with 50% w/w deuteriohypophosphorous acid afforded 1-methylnaphthalene-2-*d* which was converted to 1-naphthaldehyde-2-*d* using a synthesis of 2-naphthaldehyde described by Doukas.[25] 1-Naphthaldehyde-2-*d* (5 mg) and 6-methoxycoumaran-3-one (5 mg) were dissolved in methanol (0.2 cm^3), piperidine (10 μl) added, and the whole set aside for 24 h. During this period the solvent was allowed to evaporate. The residue was dissolved in benzene (10 cm^3) and evaporated under reduced pressure, which process was repeated twice more to ensure complete removal of the methanol. Chromatography of the residue on silica gel (5 g) with benzene afforded pure title compound (5.5 mg), m.p. 174–176 °C. Isotopic analysis: d_0, 7.3; d_1, 92.7%.

(*E*)-(*c′*)-Benzo-6-methoxyaurone was prepared from 2-naphthaldehyde (300 mg) and 6-methoxycoumaran-3-one (300 mg) using the method given for the preparation of (*E*)-6-methoxy-2′-nitroaurone. Pure title compound (246 mg) crystallized from ethanol as pale yellow, woolly needles, m.p. 172–174 °C. (Found: C, 79.5; H, 4.7. $C_{20}H_{14}O_3$ requires C, 79.45; H, 4.7%).

(*E*)-6-Methoxy-2-(2-naphthylidene-1-*d*)-coumaran-3-one. Using the procedure described for the preparation of naphthalene-1-*d*, 1-bromo-2-methylnaphthalene was converted to 2-methylnaphthalene-1-*d*. This was converted to 2-naphthaldehyde-1-*d* using Doukas[25] procedure for the preparation of 2-naphthaldehyde. 6-Methoxycoumaran-3-one (85 mg) and naphthaldehyde (85 mg) were dissolved in ethanol (1 cm^3) and piperidine (1 drop) was added. The work-up procedure used was that given above for the preparation of (*E*)-(*c′*)-benzo-6-methoxyaurone. The title compound (71 mg) crystallized from ethanol as pale yellow needles, m.p. 173–175 °C. Isotopic analysis: d_0, 2.2; d_1, 97.8%.

(*E*)-(*b′*, *e′*)-Dibenzo-6-methoxyaurone was prepared from anthra-9-aldehyde (200 mg) and 6-methoxycoumaran-3-one (164 mg) dissolved in hot absolute ethanol (4 cm^3) containing piperidine (0.4 cm^3). After standing at room temperature for 3 h, the product was collected on a filter, washed with a small quantity of ethanol, and recrystallized from benzene to yield title compound (98 mg), as orange prisms, m.p. 244–246 °C. (Found: C, 81.85; H, 4.5. $C_{24}H_{16}O_3$ requires C, 81.8; H, 4.6%).

(*E*)-3′,5′,6-Trimethoxyaurone. 3,5-Dimethoxybenzoic acid prepared by oxidation of orcinol dimethyl ether was methylated with diazomethane in the usual manner. The methyl ester was oxidized to 3,5-dimethoxybenzaldehyde via the 4-toluenesulphonyl hydrazide. 6-Methoxycoumaran-3-one (300 mg) and 3,5-dimethoxybenzaldehyde (200 mg) were converted to the title compound (237 mg) which was obtained as pale yellow needles (m.p. 159–160 °C) after recrystallization from ethanol. (Found: C, 69.4; H, 5.1. $C_{18}H_{16}O_5$ requires C, 69.2; H, 5.2%).

(*E*)-3′,5′,6-Trimethoxyaurone-4-*d*. Over a period of 0.75 h, 3,5-dimethoxytoluene (5.0 g) in sodium dried ether (25 cm^3) was added to a stirred solution of *n*-butyl lithium in hexane, which was kept under a nitrogen atmosphere. When the addition had been completed, the reaction mixture was stirred for 1 h.

Deuterium oxide (5.0 cm^3) and brine were added successively, and the organic layer removed. The aqueous layer was extracted with ether (25 cm^3) and the latter combined with the organic layer. The solvents were removed by fractional distillation. As low voltage mass spectrometric analysis of the residue showed only a 75% incorporation of deuterium had been achieved, the substrate was reacted with a further quantity of *n*-butyl lithium as described above. The yield of 3,5-dimethoxytoluene-4-*d* was almost quantitative. Isotopic analysis: d_1, 100%. It was converted to the title compound as above.

2-Cinnamylidene-6-methoxycoumaran-3-one. Cinnamaldehyde (300 mg) and 6-methoxycoumaran-3-one (300 mg) were dissolved in warm absolute ethanol (4.0 cm^3) and piperidine (0.30 cm^3) was added. After standing at room temperature for 13.3 h, the yellow precipitate was collected on a filter, washed with a small quantity of cold ethanol and recrystallized from ethanol. Pure 2-cinnamylidene-6-methoxycoumaran-3-one (373 mg) was obtained as yellow needles, m. p. 188–190 °C. (Found: C, 77.5; H, 5.1. $C_{18}H_{14}O_3$ requires C, 77.7; H, 5.1%). NMR: δ 3.88 (s, CH_3O), 6.7 (mult., olefinic H) and 7.4 (mult., aromatic H).

2-(Cinnamylidene-2,3,4,5,6-d_5)-6-methoxycoumaran-3-one. Cinnamic acid-2,3,4,5,6-d_5 was prepared from benzaldehyde-2,3,4,5,6-d_5 (see preparation of (*E*)-6-methoxyaurone-2′,3′,4′,5′,6′-d_5) and malonic acid using a well established procedure. The labelled cinnamic acid was converted to cinnamaldehyde-2,3,4,5,6-d_5 via the anilide using Mossetig's method.[26] The title compound was prepared from 6-methoxycoumaran-3-one (100 mg) and cinnamaldehyde-2,3,4,5,6-d_5 (100 mg) using the method given for the preparation of the

Table 4. Mass spectral data

5 *m/z* 252([M]$^{+\cdot}$, 56%), 251(100), 237(4), 236(13), 209(6), 208(7.5), 152(6), 150(3), 122(6), 106(6), 105(7).

6 *m/z* 251(3%), 236(2), 208(4), 180(3), 179(2), 152(7.5), 151(22), 150(100), 122(30), 119(35), 107(9).

7 *m/z* 297([M]$^{+\cdot}$, 100%), 296(76), 281(2.5), 280(8.5), 251(28), 250(62), 236(11), 235(3), 208(27), 180(7), 179(2.5), 152(15), 151(5), 150(7.5), 122(6), 107(4), 106(12).

8 *m/z* 297([M]$^{+\cdot}$, 100%), 296(76), 281(4), 280(18.5), 267(3.5), 266(3), 251(33), 250(86), 239(3), 238(2), 236(8), 235(2.5), 209(3), 208(16), 196(3.5), 180(7), 179(8.5), 152(13), 151(6), 150(7), 122(7), 107(5.5), 106(9).

9 *m/z* 282([M]$^{+\cdot}$, 29.5%), 281(2), 252(18), 251(100), 236(8), 208(3), 151(6), 141(5), 131(4), 106(4).

10 *m/z* 282([M]$^{+\cdot}$, 85%), 281(100), 267(8), 266(5.5), 252(5.5), 251(33), 239(7.5), 238(3.5), 236(3), 224(3), 196(2), 150(4), 141(5.5), 132(3.5), 122(3), 107(2.5), 106(3), 102(2).

11 *m/z* 282([M]$^{+\cdot}$, 100%), 281(72), 268(2), 267(11), 266(6), 255(3), 252(4.5), 251(12), 240(2.5), 239(14), 238(2), 224(5), 196(4.5), 168(3), 152(2), 151(2), 150(5), 143(3.5), 141(5.5), 139(2.5), 133(2), 132(24), 122(3.5), 117(3.5), 107(2), 106(3), 105(2).

12 *m/z* 266([M]$^{+\cdot}$, 100%), 265(34), 251(40), 250(6.5), 249(40), 238(3), 237(2), 236(4.5), 234(3), 223(3), 222(2), 218(3), 208(3.5), 178(3), 165(5), 152(12.5), 151(93), 150(5), 143(3), 142(2.5), 133(2.5), 132(5), 122(4), 116(16), 115(18), 108(3), 107(5), 106(5), 104(2.5), 103(5).

13 *m/z* 266([M]$^{+\cdot}$, 94%), 265(100), 252(13), 251(75), 250(6.5), 249(2), 236(5), 223(5), 222(4.5), 208(3), 165(3), 152(3.5), 151(5.5), 150(8.5), 143(2), 133(3), 132(8), 122(7), 116(3), 115(7), 107(5), 106(5.5), 104(3), 103(4.5).

14 *m/z* 266([M]$^{+\cdot}$, 91%), 265(100), 252(9), 251(51), 250(6.5), 245(2), 237(3), 236(4), 229(2), 223(5), 222(3), 163(3), 152(2.5), 151(3), 150(9.5), 147(6), 135(5), 133(5), 126(4), 122(5), 116(2), 115(6.5), 107(3), 106(3.5), 105(3), 104(3.5).

15 *m/z* 288(3), 286([M]$^{+\cdot}$, 8.5%), 252(18), 251(100), 236(15), 208(10).

16 *m/z* 288(27), 287(52), 286([M]$^{+\cdot}$, 78%), 285(100), 272(3), 270(9.5), 251(22), 243(4), 242(3), 236(5), 208(3), 152(3.5), 151(2), 150(9), 126(2), 125(4.5), 122(7.5), 107(4.5), 106(5).

17 *m/z* 288(30), 287(49), 286([M]$^{+\cdot}$, 92%), 285(100), 272(2.5), 270(5.5), 251(22), 243(5.5), 236(4.5), 208(3), 152(4.5), 151(3), 150(2), 143(2), 136(2), 126(3), 125(5), 124(3), 122(9.5), 107(6), 106(7.5).

18 *m/z* 268([M]$^{+\cdot}$, 84%), 267(16), 252(8), 251(44), 236(3), 225(3.5), 168(2), 152(2.5), 151(29), 150(100), 147(3), 145(4.5), 134(20), 122(14), 120(3), 119(3.5), 118(47), 107(5.5), 106(7).

19 *m/z* 268([M]$^{+\cdot}$, 78%), 267(100), 252(16), 251(31), 240(4), 239(14), 236(13), 225(16), 224(12), 208(2), 197(4.5), 196(4.5), 168(3), 152(2.5), 151(8), 150(6), 145(2.5), 141(3), 140(2.5), 139(4), 134(6), 122(14), 121(2), 120(2.5), 119(2), 118(3.5), 115(3), 107(13), 106(19), 105(13).

20 *m/z* 268([M]$^{+\cdot}$, 100%), 267(98), 252(10), 251(8), 240(5), 239(2), 236(2), 225(12), 224(5.5), 168(2.5), 134(4.5), 122(7), 118(7), 106(7.5), 105(3).

21 *m/z* 267([M]$^{+\cdot}$, 24%), 266(4.5), 251(3), 250(14), 249(2), 235(6), 210(3), 207(5), 169(2), 152(4.5), 151(38), 144(11), 143(8.5), 118(11), 117(100), 116(8.5), 115(8), 107(9), 104(11).

22 *m/z* 267([M]$^{+\cdot}$, 100%), 266(93), 252(11), 251(44), 239(3), 236(3), 224(6), 223(4.5), 151(5), 117(15), 106(4), 104(4.5).

23 *m/z* 267([M]$^{+\cdot}$, 100%), 266(35), 251(4), 224(4.5), 223(2), 207(3), 196(2), 151(3), 117(19), 106(8), 104(3).

24 *m/z* 302([M]$^{+\cdot}$, 100%), 301(83), 286(6), 285(13), 273(2), 270(2), 259(4), 258(4.5), 203(3.5), 202(7), 179(5), 153(8), 152(72), 151(15), 150(7), 140(6.5), 139(12), 122(4), 107(4), 106(4).

25 *m/z* 302([M]$^{+\cdot}$, 100%), 301(95), 286(7), 285(2), 259(6), 258(3.5), 202(6), 153(3), 152(21), 150(6), 140(5), 139(8), 122(3), 107(2), 106(4.5).

26 *m/z* 352([M]$^{+\cdot}$, 81%), 351(42), 336(3), 335(16), 323(2), 320(2), 308(2), 252(3.5), 229(4), 203(18), 202(100), 201(9), 200(7), 189(8.5), 178(4), 176(7), 147(3).

27 *m/z* 278([M]$^{+\cdot}$, 100%), 277(65), 263(10), 262(5), 261(8), 249(6), 235(4), 234(6.5), 228(4.5), 202(3), 201(17), 189(4.5), 186(3), 179(4), 178(5), 163(3), 155(6), 152(2.5), 151(26), 150(9), 147(5), 139(11), 128(5), 127(36), 126(7), 122(4), 115(10), 107(2.5), 106(4), 102(3).

unlabelled analogue. The pure product (35 mg) crystallized from ethanol as yellow needles, m.p. 185–187 °C. The yield was low due to spillage. Isotopic analysis: d_4, 3.5; d_5, 96.5%.

2-Cinnamylidene-6-methoxycoumaran-3-one-carbonyl-^{18}O. 6-Methoxycoumaran-3-one-carbonyl-^{18}O (2.5 mg) (see above) and cinnamaldehyde (3.0 μl) were dissolved in absolute ethanol (25.0 μl) and piperidine (0.2 μl) was added. After standing at room temperature for 4 h, the volatile materials were removed under reduced pressure. Pure title compound was obtained by preparative TLC (benzene). Isotopic analysis showed that ^{18}O had been incorporated to the extent of 45.7%.

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