Phytochemistry, 1973, Vol. 12, pp. 879 to 889. Pergamon Press. Printed in England.

APPLICATION OF DEUTERIATION BY GLC IN THE IDENTIFICATION OF FUKINONE IN *PETASITES HYBRIDUS*

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(*Received* 15 *October* 1972. *Accepted* 8 *November* 1972)

Key Word Index—*Petasites hybridus*; Compositae; sesquiterpenoids; fukinone; deuterium–hydrogen exchange; GC–MS.

Abstract—A sesquiterpenoid ketone was isolated from leaf extracts of *Petasites hybridus*. The available sample (50 μg) was characterised from MS data obtained by deuterium labelling *in transitu* in the GLC column, and was identified as fukinone, except that optical homogeneity was not established.

IN THE course of an investigation[1] of the biosynthesis of petasin in *Petasites hybridus* L. (Compositae),[2] the lipid-soluble extract of the leaves was examined for the presence of potential biosynthetic intermediates.[3] Group separation procedures employing specially developed methods of gel partition chromatography[3–5] yielded one particular fraction containing four components, which appeared to be sesquiterpenoid ketones or lactones on the basis of their chromatographic behaviour.[3,4] A preliminary examination by combined GC–MS led to their tentative formulation as three ketones and one lactone (Table 1).

TABLE 1. COMPONENTS OF THE 'KETONE AND LACTONE' FRACTION FROM *P. hybridus* LEAF EXTRACTS

Compound	Retention index 1% OV-1	Molecular ion	Tentative formula	Quantity* (μg)
A	1720	220	$C_{15}H_{24}O$	100
B	1725	218	$C_{15}H_{22}O$	600
C	1760	—	—	10
D	1770	234	$C_{15}H_{22}O_2$	8

* Recovered from 150 g fr. wt of leaf tissue: amounts of A, B and C were estimated by gas chromatography.

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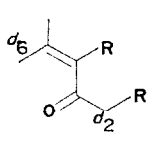
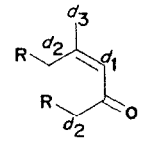
[1] BROOKS, C. J. W. and KEATES, R. A. B. (1972) *Phytochem.* **11**, 3235.
[2] AEBI, A., BÜCHI, J., WAALER, T., EICHENBERGER, E. and SCHMUTZ, J. (1955) *Pharm. Acta Helv.* **29**, 277.
[3] KEATES, R. A. B. and BROOKS, C. J. W. in preparation.
[4] ELLINGBOE, J., NYSTRÖM, E. and SJÖVALL, J. (1970) *J. Lipid Res.* **11**, 266.
[5] BROOKS, C. J. W. and KEATES, R. A. B. (1969) *J. Chromatog.* **44**, 509.

The identification of ketone-B and lactone-D was completed by conventional means,[3] but the limited quantity of compound-A (50 μg after purification) required special techniques for its structural elucidation. The presence of an α,β-unsaturated ketone was indicated by the UV absorption (λ_{max} 249 nm, ϵ_{max} 8000 in EtOH).



(I)



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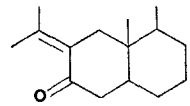
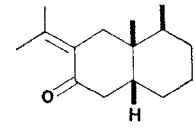


(III)



(IV)

The development of techniques for deuteriation of carbonyl compounds during GLC has provided a method by which μg quantities of sample may be labelled to a high degree of isotopic purity.[6–10] This was applied to compound-A, using GC–MS for direct analysis of the product of deuterium exchange. The major fragment ions are tabulated in Table 2,

TABLE 2. TABULATION OF MASS SPECTRA OF COMPOUND A, RECORDED AT 20 eV, WITH AND WITHOUT DEUTERIUM EXCHANGE LABELLING

	Relative	Deuterium substitution*								
m/e	intensity	d_8	d_7	d_6	d_5	d_4	d_3	d_2	d_1	d_0
220	78	78	—	—	—	—	—	—	—	—
205	8	4	—	—	4	—	—	—	—	—
177	7	3	—	—	3	—	—	1	—	—
152	6	—	—	—	—	—	—	3	3	—
149	12	—	—	9	—	—	—	4	—	—
123	12	—	—	—	—	—	—	6	6	—
111	22	12	—	—	—	—	—	—	—	9
110	41	17	—	—	—	—	—	—	—	24
109	100	—	—	—	—	—	—	—	—	100
108	22	—	—	—	—	—	—	—	—	22
96	44	—	—	44	—	—	—	—	—	—
95	13	—	—	6	—	—	—	—	—	7
83	6	—	—	6	—	—	—	—	—	—
82	8	—	—	8	—	—	—	—	—	—
81	8	—	—	—	—	—	—	—	—	8
68	28	—	—	28	—	—	—	—	—	—

* These values were adjusted to 100% saturation by deuterium. The deuterium substitution of the molecular ion indicated an abundance of protium of 5% in the enolisable positions: contributions to the molecular ion were: d_8, 51%; d_7, 41%; d_6, 8%. Assuming protium to be equally distributed in all eight positions, the expected contributions to a true d_6 fragment were calculated: d_6, 66%; d_5, 30%; d_4, 4%. Observed d_8 and d_6 fragments having this distribution of satellite ions were reported as d_8 and d_6 respectively, the relative abundance of the peak being adjusted by summation of the contributions due to the satellite ions. True d_5 fragments in the presence of d_6 would be indicated by abnormally high d_4 intensity.

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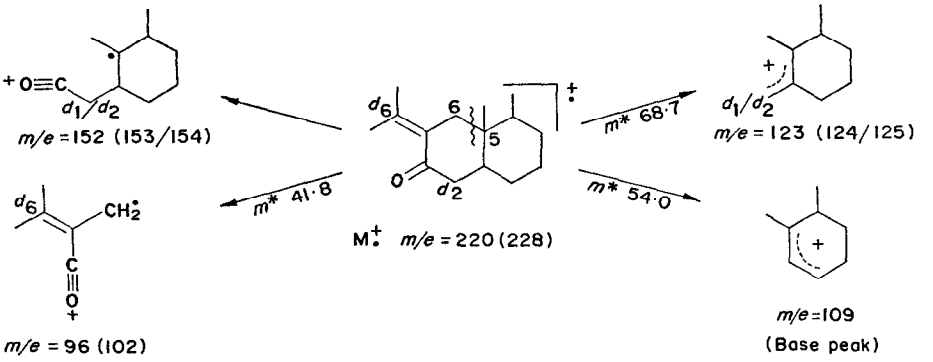
[8] ELIAS, H. (1968) in *Advances in Chromatography* (edited by GIDDINGS, J. C. and KELLER, R. A.) Vol. 7, p. 243, Edward Arnold, London.
[9] ANTHONY, G. M. and BROOKS, C. J. W. (1970) *Chem. Commun.* 200.
[10] ANTHONY, G. M. and BROOKS, C. J. W. (1971) *Gas Chromatography* 1970 (edited by STOCK, R.), p. 70, Institute of Petroleum, London.

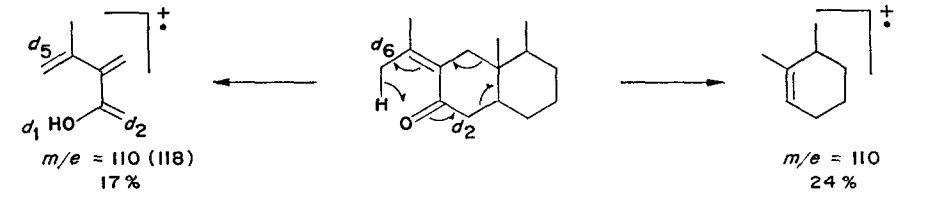
and their respective mass shifts on deuteriation are indicated. The molecular ion of compound-A was increased by 8 m.u. This high level of deuteriation could only be obtained from an α,β-unsaturated ketone of partial structure I or II. The former was strongly indicated by the abundance of fragments corresponding to d_6 or d_2 substitution (Table 2) and the comparative absence of d_3, d_4 and d_5 fragments.

On chemotaxonomic grounds,[11] we fitted the data to an eremophilane structure. Formula III was the only arrangement that permitted d_8-substitution, and was, therefore, assigned tentatively to compound A. Fukinone (IV), having *cis*-stereochemistry at the ring junction, had already been isolated from *Petasites japonicus* Maxim.[12]



SCHEME 1. PRINCIPAL FRAGMENTS IN MS OF THE SESQUITERPENOID KETONE, WITH AND WITHOUT DEUTERIUM LABELLING.

The MS of compound-A and its deuteriated analogue were interpretable in terms of the structure of fukinone. The lack of incorporation of deuterium into the base peak, *m/e* 109, indicated that this was the hydrocarbon fragment from ring-A depicted in Scheme 1. Fragmentation appeared to be directed by the cleavage of the bond between C-5 and C-6 as shown, this being β with respect to the double bond and γ to the carbonyl function. Further bond fissions led to four of the abundant ions of the MS. Ions having *m/e* 110 were coincidentally half the mass of the molecular ion, *m/e* 220. Deuteriation demonstrated that both hydrocarbon and carbonyl-containing fragments contributed to this peak. A rearrangement analogous to the McLafferty type, but with a 10-membered transition state, might account for the formation of these radical ions (Scheme 2).



SCHEME 2. PROPOSED ORIGIN OF PEAKS AT *m/e* 110 IN THE MS OF THE SESQUITERPENOID KETONE.

[11] HEROUT, V. and ŠORM, F. (1969) in *Perspectives in Phytochemistry* (edited by HARBORNE, J. B. and SWAIN, T.), p. 139, Academic Press, London.

[12] NAYA, K., TAGAKI, I., KAWAGUCHI, Y., ASADA, Y., HIROSE, Y. and SHINODA, N. (1968) *Tetrahedron* **24**, 5871.

The foregoing considerations strongly favoured the identification of compound-A as fukinone: comparison with an authentic sample by GC–MS confirmed the identity of chromatographic properties and MS fragmentations.

EXPERIMENTAL

The procedures leading to the isolation of compound-A are described elsewhere.[3,13]

Deuteriation by GLC. Deuterium labelling was carried out on the column by GLC,[6,8] using the stationary phase 1% Apiezon L/0 · 5% $Ba(OH)_2$, activated by pre-injection with deuteriomethanol, MeOD.[9,10]

MS. MS were obtained using an LKB 9000 GC–MS apparatus. The deuterium-labelled sample was prepared using an Apiezon L-$Ba(OH)_2$ column directly in this instrument, and was analysed without further isolation.

Acknowledgements—We are grateful to Professor K. Naya (Kwansei University, Japan) for a gift of authentic fukinone, and to Mrs. J. Borthwick for assistance with GC–MS measurements. The LKB 9000 instrument was purchased under SRC grant No. B/SR/2398. The work was performed during the tenure of a Demonstratorship (R.A.B.K.) and of a research grant from the MRC (C.J.W.B.).

[13] KEATES, R. A. B. (1970) Ph.D. Thesis, University of Glasgow.