Tetrahedron Letters No. 24, pp. 2157-2160, 1971. Pergamon Press. Printed in Great Britain.

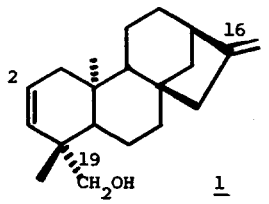
A GIBBANE METABOLITE FROM (-)-KAURA-2,16-DIEN-19-OL

BY I.F. Cook, P.R. Jefferies and J.R.Knox

Department of Organic Chemistry, University of W.A.,Nedlands, Western Australia.

(Received in UK 29 April 1971; accepted in UK for publication 13 May 1971)

Gibberellins have a characteristic plant growth promoting activity which, with few exceptions, is only otherwise observed with various gibbane derivatives and with diterpenes which have been indicated to be intermediates of normal gibberellin biosynthetic pathways.[1,2] One exception is (-)-kaura-2,16-dien-19-ol (1)[3]. We have examined the metabolism of this compound by Gibberella fujikuroi, a fungal source of gibberellins, and have observed the formation of a gibbane metabolite (2) which may be responsible for the bioactivity shown by the dienol (1) in dwarf corn assays[3].



1

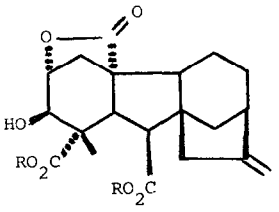
The metabolite was isolated as the gummy dimethyl ester (3), (m/e 404, M^+). Although the molecular ion abundance was too low for a satisfactory high resolution measurement, the precise mass of the M- CH_4O ion (372.156) is consistent with the molecular formula $C_{22}H_{28}O_7$. The i.r. spectrum of 3 has absorptions due to hydroxy (3620^{-1}) ester ($1725cm^{-1}$) and γ-lactone ($1780cm^{-1}$) functionalities.

Upon oxidation of the metabolite diester with Jones reagent the crystalline ketone (4) $C_{22}H_{26}O_7$ (m/e 402.166) m.p. 142-4°, $[\alpha]_D$ -76° was obtained. As required by the structural assignment it lacks hydroxyl absorption in the infrared spectrum; however, the ketone absorption occurs with that of the ester near $1725cm^{-1}$.

TABLE I : NMR Spectra (τ)

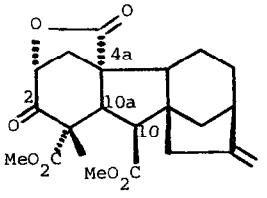
Compound (Solvent)	CO_2Me	1-Me	H-3	H-10#	H-10a#	$=CH_2$
3* ($CDCl_3$)	6.35, 6.30	8.74	5.35	6.34	7.34	5.12
(C_5D_5N)	6.39, 6.30	8.45	**	5.95	6.99	5.08
4 ($CDCl_3$)	6.25, 6.30	8.50	5.30Ø	6.07	7.45	5.00, 5.13

* H-2 Obscured, # J = 12Hz, Ø J = 7Hz, ** obscured

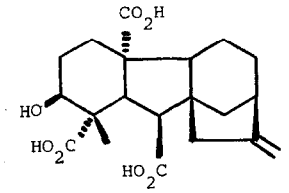


2 R = H

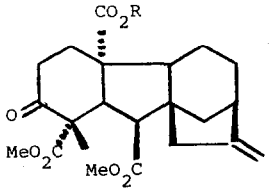
3 R = Me



4

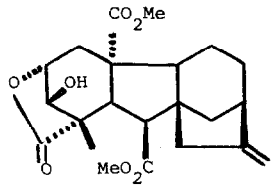


5

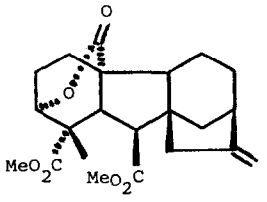


6 R = Me

8 R = Et



7



9

Table I presents a summary of the n.m.r. spectra of 3 and 4 which each give signals characteristic of a tertiary methyl, an exocyclic methylene functionality and two methyl ester groupings. Not unexpectedly, H-3 gives an unresolved signal ($W_{\frac{1}{2}}$=4Hz) in the spectra of 3 but with 4 it appears as a clean doublet. The multiplicity of the signal in the latter case reflects not only the loss of coupling with H-2 because of the new oxidation state but also, apparently, the absence of coupling to H-4ß due to an approximately 90° dihedral angle in the preferred conformation of the A-ring of 4.

For both compounds the 10- and 10a- protons give rise to an AB pattern which is characteristic of gibberellins[4]. The 10a-proton of 3 in $CDCl_3$ solution resonates at a low field position which is similar to that of other 2ß- hydroxylated gibberellins[5,6]. Furthermore with a C_5D_5N solution this signal and that of the tertiary methyl group undergo downfield shifts the magnitudes of which are typical of 2ß-hydroxylated compounds[5,6].

Chemical evidence for the structure of the metabolite diester came from a chemical correlation with gibberellin A_{13} (5). For this purpose the lactone functionality of the ketone (4) was reductively opened by treatment with $Cr(OAc)_2$ in THF giving an acidic material. The product from further methylation (CH_2N_2) was found to be identical with an authentic sample of the keto-trimethyl ester (6) previously prepared from gibberellin A_{13}[7].

This transformation together with the spectral data presented above indicates two structural possibilities (3 and 7) for the metabolite diester. They have been differentiated in the following way:

The acidic fraction from the $Cr(OAc)_2$ reduction was treated with ethyl iodide and Na_2CO_3 in acetone to give an ethyl ester (8) (m/e 432, M^+). By reduction of this material with $NaBH_4$ followed by mild treatment with HOAc a product was obtained which was shown to be identical with the product (9) from $NaBH_4$ reduction of the ketotrimethyl ester (6)[7]. This clearly demonstrates that the γ-lactone functionality of the metabolite diester involves the C=O group attached at C-4a as in structure (3).

Considerable difficulties were encountered in attempts to isolate the metabolite as the free acid (2) rather than the dimethyl ester (3). However a small amount of 2 was obtained in a homogeneous, although gummy, form; upon methylation it gave only the dimethyl ester (3). A bioassay against d_1-maize showed a significant response (leaf sheath extension as a percentage of controls: 0.1 µg, 140%; 1µg, 190%; 10µg, 280%) which is comparable with that observed with gibberellins A_5, A_6, A_{18} and A_{22}.[1,8,9,10]

ACKNOWLEDGEMENTS

We thank Dr. A. McComb for bioassays, Dr. A. Duffield for high resolution mass spectra and Dr. R.H.B. Galt for a sample of the ketotrimethyl ester (6). This work was supported by a grant from the Australian Research Grants Committee.

REFERENCES

1. A. Crozier, C.C. Kuo, R.C. Durley and R.P. Pharis, Can. J. Bot., 48, 867 (1970).
2. P.W. Brian, J.F. Grove, T.P.C. Mulholland, Phytochemistry, 6, 1475 (1967).
3. E.L. Ghisalberti, P.R. Jefferies and E.J. Middleton, Aust. J. Chem., 22, 455 (1969).
4. N. Sheppard, J. Chem. Soc., 3040 (1960).
5. J.R. Hanson, J. Chem. Soc., 5036 (1965).
6. K. Koshimizu, H. Fukui, M. Inui, Y. Ogawa and T. Mitsui, Tetrahedron Letters, 1143 (1968).
7. R.H.B. Galt, J. Chem. Soc., 3143 (1965).
8. P.W. Brian, H.G. Hemming and D. Lowe, Ann Bot. (London), 28, 369 (1964).
9. N. Murofushi, N. Takahashi, T. Yokota, J. Kato, Y. Shiotani and S. Tamura, Agr. Biol. Chem., 33, 592 (1969).
10. M. Katsumi, Y. Ogawa and K. Koshimizu, Bot. Mag. (Tokyo), 81, 491 (1968).