remaining from the hexane extraction were treated with EtOAc in a similar way and then chromatographed on silica gel. The fraction eluted with hexane-EtOAc (4:1) (940 mg) was acetylated with Ac_2O–pyridine at room temp. followed by purification on a silica gel column with hexane–Et_2O, affording 257 mg of the diacetyl derivative of **6**.

Chamisellin (**1a**). ^{13}C NMR (20.15 MHz, $CDCl_3$): 126.8 (C-1), 23.0, 24.2 (C-2), 35.9, 38.5 (C-3), 135.8 (C-4), 129.6, 131.9 (C-5), 70.2 (C-6), 50.0, 51.2, 53.9 (C-7), 78.7, 79.8, 83.3 (C-8), 42.3, 47.2 (C-9), 130.4 (C-10), 135.8 (C-11), 170.1 (C-12), 126.8 (C-13), 16.6, 20.7 (C-14), 17.5 (C-15).

Chemissanthin (**2a**). ^{13}C NMR (20.15 MHz, $CDCl_3$); δ129.5 (C-1), 25.8 (C-2), 39.0 (C-3), 127.4 (C-5), 77.1 (C-6), 54.7 (C-7), 71.3 (C-8), 52.8 (C-9), 169.3 (C-12), 126.6 (C-13), 17.2 (C-14), 17.7 (C-15).

4β,5α-Epoxi-6α-hydroxygermacra-1(10),11(13)-dien-8α,12-olide (**3**). ^{13}C NMR (20.15 MHz, $CDCl_3$): δ 127.2 (C-1), 23.4 (C-2), 37.3 (C-3), 61.3 (C-4), 64.7 (C-5), 68.8 (C-6), 46.0 (C-7), 77.1 (C-8), 43.2 (C-9), 129.9 (C-10), 124.6 (C-11), 169.6 (C-12), 127.8 (C-13), 15.9 (C-14), 19.3 (C-15).

Diacetyl derivative of **6**. $[\alpha]_D - 75.1°$ ($CHCl_3$; *c* 1.0); MS (probe) 70 ev, *m/z* (rel. int.): 306 $[M-CH_2=C=O]^+$ (1), 289 $[M-MeCOO]^+$ (5), 288 $[M-AcOH]^+$ (2), 246 $[M-AcOH-CH_2=C=O]^+$ (30), 228 $[M-AcOH-AcOH]^+$ (80), 213 (50); ^1H NMR (80 MHz, $CDCl_3$): δ 4.75–5.05 (3H, *m*, H-1, H-5, H-6), 2.9 (1H, *m*, H-7), 4.03 (1H, *m*, H-8), 5.74 (1H, *dd*, 1, 2.5, H-13), 6.23 (1H, *dd*, 1, 2.5, H-13'), 1.79 (3H, *br s*, H-14), 5.16 (2H, *m*, H-15), 1.98 (3H, OAc), 2.0 (3H, OAc).

Acknowledgements—We thank Professor F. Valle (Departamento de Botánica de la Facultad de Ciencias de la Universidad de Granada) for the identification of the plant material.

REFERENCES

1. Bohlmann, F. and Zdero, C. (1973) *Chem. Ber.* **106**, 845.
2. Pascual Teresa, J. de, Gonzalez, M. S., Dios, M. A. de, San Segundo, J. M. and Bellido, I. S. (1981) *Riv. Ital. E.P.P.P.S.* **62**, 355.
3. Yoshishara, K., Yoshimoto, O. and col. (1969) *Tetrahedron Letters* 263.
4. Shafizadeh, F. and Bhadane, N. R. (1973) *Phytochemistry* **12**, 857.
5. Geissman, T. A., Lucas, A. J. and Saitoh, T. (1973) *Biochem. Syst. Ecol.* **1**, 13.
6. Bohlmann, F., Jakupovic, J., Ahmed, M. and Schuster, A. (1983) *Phytochemistry* **22**, 1623.
7. Bohlmann, F., Adler, A., Jakupovic, J., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 1349.
8. Horst, H. A. and Linde, M. S. R. (1967) *Helv. Chim. Acta* **50**, 1961.

Phytochemistry, Vol. 27, No. 12, pp. 3970–3972, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00
Pergamon Press plc.

[1β,2β,3β-$^3H_3$]GIBBERELLIN A_{20}: CONFIRMATION OF STRUCTURE BY ^3H NMR AND BY MASS SPECTROMETRY

CHRISTINE L. WILLIS, PAUL GASKIN and JAKE MACMILLAN

AFRC Research Group, Department of Organic Chemistry, The University of Bristol, Bristol BS8 1TS, U.K.

(*Received* 19 *April* 1988)

Key Word Index—Gibberellin A_{20}; tritiogenation of gibberellin A_5; tritium-labelling; regio-selectivity; ^3H NMR.

Abstract—[1β,2β,3β-$^3H_3$]Gibberellin A_{20}, 55 Ci/mmol, has been prepared by catalytic reduction of gibberellin A_5 methyl ester 13-acetate 16,17-epoxide, followed by deoxygenation of the epoxide and aqueous alkaline hydrolysis. The regio- and stereo-selectivity of labelling has been established by NMR and mass spectrometry.

INTRODUCTION

Gibberellin A_{20} (**1**), stereospecifically labelled with tritium at the 2β- and 3β- positions, was required for studies with enzymes that catalyse the 2β- and 3β-hydroxylation and 2,3-didehydrogenation of gibberellin A_{20} (GA_{20}) (**1**). Murofushi *et al.* [1] have prepared GA_{20} (**1**) by catalytic hydrogenation of GA_5 methyl ester (**3**) but found it difficult to separate GA_{20} methyl ester (**2**) from the complex mixture of reduction products. To avoid this problem, Murofushi *et al.* [2] protected the 16,17-double bond in GA_5 methyl ester by forming the 16,17-epoxide (**5**), then reduced the 2,3-double bond catalytically with a mixture of tritium and hydrogen gas. Deoxygenation of the reduced epoxide by the method of Cornforth *et al.* [3], followed by hydrolysis of the methyl ester with aqueous sodium hydroxide gave tritiated GA_{20}, presumed to be [2,3-$^3H_2$]GA_{20}, but the stereo- and regio- specificity of the tritium atoms was not established. To determine the stereo- and regio-selectivity of catalytic reduction of the

2,3-ene Albone *et al.* [4] have recently investigated the reduction of GA_5 methyl ester 13-acetate 16,17-epoxide (**6**) with deuterium gas and 10% palladium on calcium carbonate. Deoxygenation of the epoxide and hydrolysis of the acetate and methyl ester groupings gave [1β,2β,3β-$^2H_3$]GA_{20}. The position and stereochemistry of the deuterium atoms was established by a combination of ^1H, ^2H and ^{13}C NMR spectroscopy. From the ^2H NMR spectrum (Fig. 1) the approximate ratio for the 1β,2β, and 3β-^2H signals was 25:37:38 and the mass spectrum (Fig. 2B) showed the incorporation of 24% $^2H_3$, 41% $^2H_2$ and 28% $^2H_1$.

Tritium-labelling of GA_{20} (**1**) was performed by Amersham International with undiluted tritium gas using the conditions described for deuterium labelling by Albone *et al.* [4]. By analogy, the product was assumed to be [1β,2β,3β-$^3H_3$]GA_{20}. However, in studies (to be reported elsewhere) on the *in vitro* enzymatic conversion of this tritium labelled GA_{20} into GA_5 (**4**), no radioactive GA_5 was detected. One possible explanation for this result was that the labelled GA_{20} did not contain a [1β-^3H] label and hence the studies now reported were undertaken.

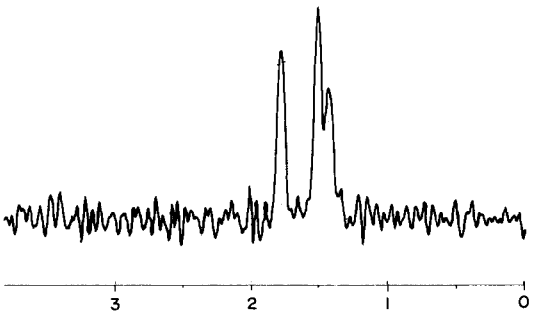


Fig. 1. ^2H NMR spectrum of [1β,2β,3β-$^2H_3$]GA_{20}.

RESULTS AND DISCUSSION

Capillary GC/MS of the MeTMSi derivative of the [^3H]GA_{20} confirmed the chemical purity and the presence of three tritium atoms. The incorporation was 17% $^3H_3$, 54% $^3H_2$, 22% $^3H_1$ and 3% $^3H_0$ from which a specific radio-activity of 55 Ci/mol was calculated using the method of Bowen *et al.* [5]. This specific activity was higher than that (40 Ci/mmol) estimated by Amersham International from the balance of the radio-activity used and recovered. The mass spectrum (Fig. 2A, C) of the MeTMSi derivatives of both the [$^3H_3$]GA_{20} and unlabelled GA_{20} contain an intense ion at *m/z* 375 which has been shown [6, 7] to arise from the loss of C-1, C-2 and C-3 with the transfer of a hydrogen atom. Thus the three tritium labels are confined to positions C-1, C-2 and C-3. The ^1H-decoupled ^3H NMR spectrum (Fig. 3) of the [$^3H_3$]GA_{20} showed three complex signals, resulting from ^3H–^3H coupling, but the chemical shifts corresponded to those of the ^2H-signals in [1β,2β,3β-$^2H_3$]GA_{20} of established stereochemistry [4]. To eliminate the ^3H–^3H couplings and thereby simplify the ^3H NMR spectrum, catalytic reduction of the available 2,3-dehydroGA_9 methyl ester 17-norketone (**7**) was performed using a 1:10 mixture of tritium and hydrogen gas. The ^3H NMR spectrum (Fig. 4) of the product (**8**) contained three singlets at the expected chemical shifts for 1β,2β and 3β-tritium atoms in the approximate ratio of 1:4:4.

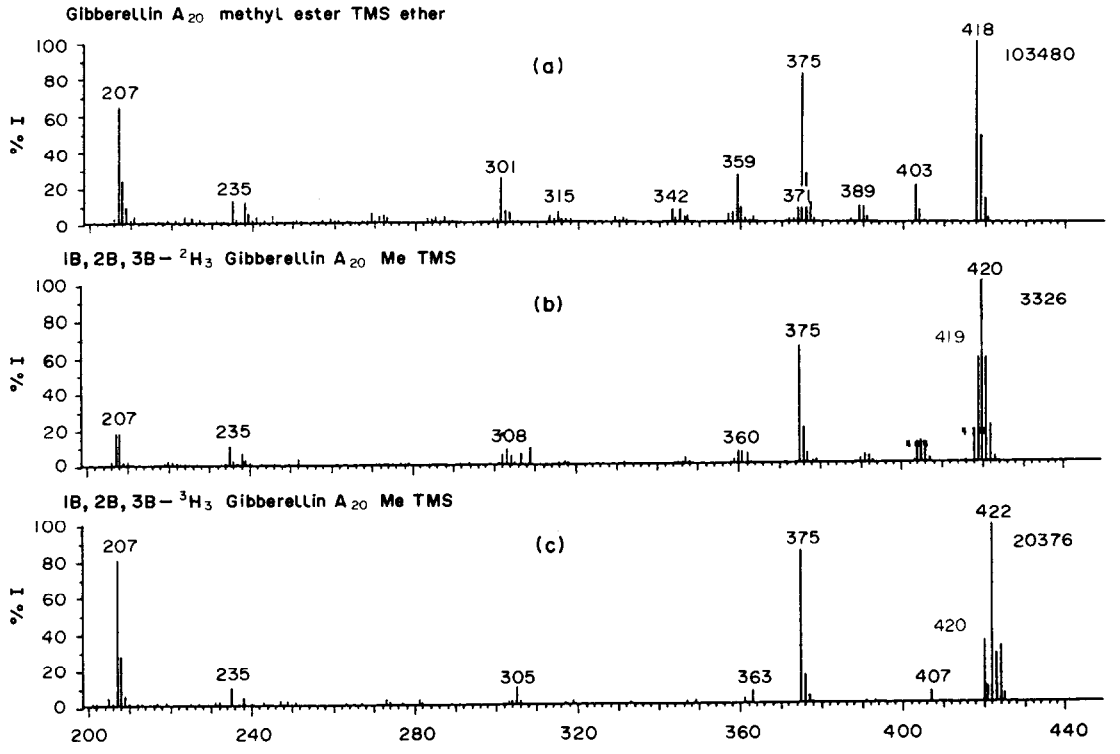


Fig. 2. GC/MS of the Me ester Me_3Si ether of: A. GA_{20}; B. [1β,2β,3β-$^2H_3$]GA_{20}; C. [1β,2β,3β-$^3H_3$]GA_{20}.

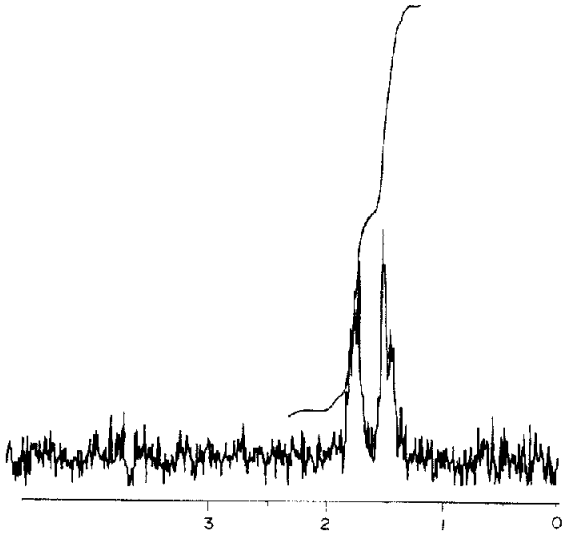


Fig. 3. ^{3}H NMR spectrum of [1β,2β,3β-$^3H_3$]GA$_{20}$ (^{1}H-decoupled).

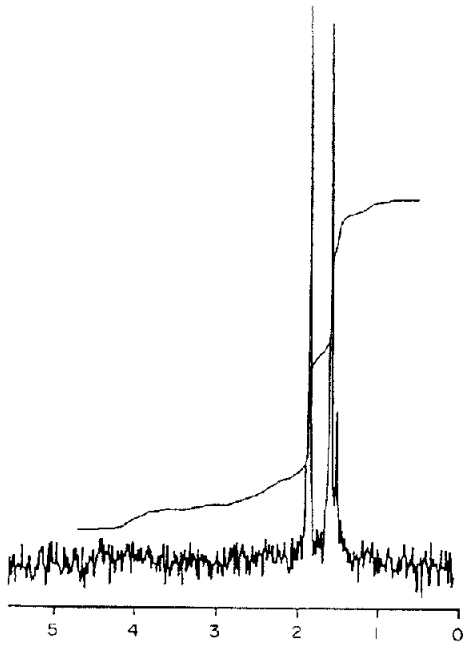
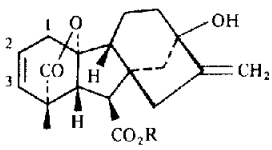
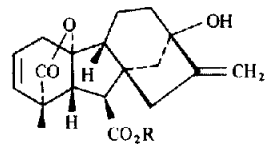


Fig. 4. ^{3}H NMR spectrum of [1β,2β,3β-$^3H_3$]GA$_9$ methyl ester norketone (**8**) (^{1}H decoupled).

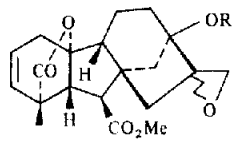
These data show that catalytic reduction of GA$_5$ methyl ester 13-acetate 16,17-epoxide (**6**) with tritium, as with deuterium, occurs exclusively from the β-face and is accompanied by the introduction of a 1β-tritium. The 1β-tritium probably results from the direct exchange of the allylic 1β-hydrogen on the surface of the catalyst; proton-deuterium exchanges have previously been noted on the surface of heterogeneous catalysts [8]. A less likely alternative is that there is isomerization of the 2-ene to the 1-ene on the catalyst, followed by reduction of the 1-ene by tritium.



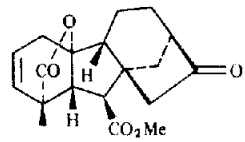
1 R=H
2 R=Me



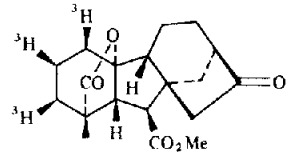
3 R=Me
4 R=H



5 R=H
6 R=Ac



7



8

EXPERIMENTAL

For GC/MS analyses: the samples were derivatized as methyl esters Me$_3$Si ethers. Deuterium NMR samples were recorded in $CHCl_3$ at 400 MHz with int. $CDCl_3$ as the reference. Tritium NMR samples were sealed in cylindrical microcells (100 μl) which were inserted into standard (5 mm) NMR tubes filled to a depth of *ca* 4 cm with CCl_4. The spectra were recorded in $CDCl_3$ 96 MHz. The chemical shifts (δ_F) were measured from int. TMS and constant field locked to the deuterium signal of $CDCl_3$.

Acknowledgement We are grateful to Dr J. R. Jones (Surrey University) for ^{3}H NMR spectra.

REFERENCES

1. Murofushi, N., Takahashi, N., Yokota, T. and Tamura, S. (1968) *Agric. Biol. Chem.* **32**, 1239.
2. Murofushi, N., Durley, R. C. and Pharis, R. P. (1974) *Agric. Biol. Chem.* **38**, 475.
3. Cornforth, J. W., Cornforth, R. H. and Mathew, K. K. (1959) *J. Chem. Soc.* 112.
4. Albone, K. S., MacMillan, J., Pitt, A. R. and Willis, C. L. (1986) *Tetrahedron* **42**, 3203.
5. Bowen, D. H., MacMillan, J. and Graebe, J. E. (1972) *Phytochemistry* **11**, 2253.
6. Takahashi, N., Murofushi, N., Tamura, S., Wasada, N., Hoshimo, H. and Tsuchiya, T. (1969) *Org. Mass Spectrom.* **2**, 711.
7. Beale, M. H., Gaskin, P., Kirkwood, P. S. and MacMillan, J. (1980) *J. Chem. Soc. Perkin 1* 885.
8. Smith, G. V. and Burwalls, Jr. R. L. (1962) *J. Am. Chem. Soc.* **84**, 925.