Origin of the Oxygen Atoms in the Lactone Bridge of C_{19}-Gibberellins

By John R. Bearder* and Jake MacMillan
(*Department of Organic Chemistry, The University, Bristol* BS8 1TS)
and Bernard O. Phinney
(*Department of Biology, University of California, Los Angeles, California* 90024)

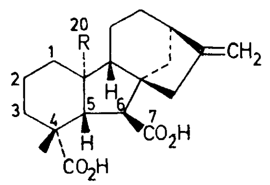
Summary [^{18}O]-Label in the 19-oic acid of the C_{20}-gibberellins, GA_{12} and GA_{12}-alcohol, is incorporated without loss into C_{19}-gibberellins, using cultures of *Gibberella fujikuroi*, mutant Bl-41a.

In the biosynthesis of the gibberellin (GA) plant hormones, the conversion of C_{20}-GAs into C_{19}-GAs is an unsolved problem. For example, in the transformation of GA_{12} (**1**) into GA_9 (**5**)[1,2] in cultures of *Gibberella fujikuroi* the compounds (**2**; 19,20-lactone), (**3**), and (**4**), representing successive oxidation at C-20, do not act as intermediates.[2] A clue is provided by the following [^{18}O]-labelling studies which indicate that both oxygen atoms in the lactone bridge of C_{19}-GAs are derived from the 19-oic acid of their C_{20}-GA precursors.

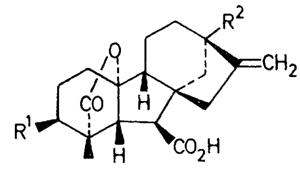
GA_{12}-alcohol (**8**)[3] was prepared with 55 atom % of [$^{18}O_1$] in the 19-oic acid by hydrolysis of the methyl ester using KO^{t}Bu–HO^{t}Bu containing $H_2{}^{18}O$ (61 atom %). Oxidation of the $[^{18}O_1]$-alcohol (**8**) gave GA_{12} (**1**) containing 56 atom % of $[^{18}O]$ in the 19-oic acid. Both $[^{18}O_1]$-acids (**1**) and (**8**) were cultured with resuspended mycelium of the fungus, *Gibberella fujikuroi* mutant Bl-41a, which is blocked for GA biosynthesis.[4] After 2 and 5 days, the $[^{18}O]$-content of the metabolites was determined by g.l.c.–m.s. of the derivatised acids, obtained by extraction of the culture medium.

$[^{18}O_1]$-GA_{12} (**1**) was metabolised to GA_{15} (**2**; 19,20-lactone), GA_{24} (**3**), GA_{25} (**4**), and GA_9 (**5**), respectively, containing 32, 53, 55, and 53 atom % of $[^{18}O]$. The ^{18}O-content was measured from the $M^+ - 15$ ion in the mass spectrum of the Me_3Si esters. Similarly $[^{18}O_1]$-GA_{12}-alcohol (**8**), known[3,5] to be an excellent precursor of GA_3 (**12**), gave GA_{14} (**9**), GA_{36} (**10**), GA_{13} (**11**), GA_4 (**7**), GA_1 (**6**), and GA_3 (**12**), respectively, containing 59, 54, 53, 54, 56, and 55 atom % of $[^{18}O]$. In these cases the $[^{18}O]$-content was determined from the $M^+ - 15$ ion in the mass spectra of the methyl ester Me_3Si ethers. The mass spectra of the C_{19}-GAs showed that the $[^{18}O]$-atoms were in the lactone ring. For example, the fragmentation ions due to the loss of Me_3SiOH and $Me_3SiOC(H){=}O$ from the M^+ ion of GA_9Me_3Si ester contained 55 atom % $[^{18}O]$.

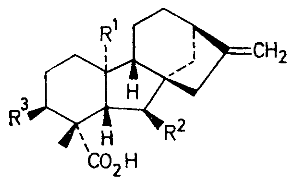
These results show that both oxygen atoms in the 19-oic acid of C_{20}-GAs are incorporated into the lactone ring of C_{19}-GAs. They also indicate that the substrates GA_{12} (**1**) and GA_{12}-alcohol (**8**), and intermediates, are not covalently bound through the 19-oic acid to the enzyme(s) catalysing the conversion and that the 19,20-lactone of (**2**) is not an intermediate. Furthermore, the conversion must involve an intermediate with an electrophilic centre at C-10 which is attacked by the 19-oic acid; an intermediate 10α-alcohol is therefore excluded.



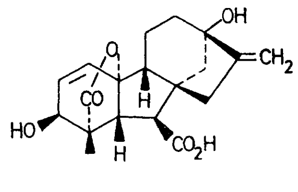
(1) R = Me
(2) R = CH_2OH
(3) R = CHO
(4) R = CO_2H



(5) $R^1 = R^2 = H$
(6) $R^1 = R^2 = OH$
(7) $R^1 = OH, R^2 = H$



(8) $R^1 = Me$, $R^2 = CH_2OH$, $R^3 = H$
(9) $R^1 = Me$, $R^2 = CO_2H$, $R^3 = OH$
(10) $R^1 = CHO$, $R^2 = CO_2H$, $R^3 = OH$
(11) $R^1 = R^2 = CO_2H$, $R^3 = OH$



(12)

We thank Mr. Paul Gaskin for the g.l.c.–m.s., and the A.R.C., N.A.T.O., and N.S.F. for financial support.

(*Received, 2nd August* 1976; *Com.* 885.)

[1] R. Evans and J. R. Hanson, *J.C.S. Perkin I*, **1975**, **663**.
[2] J. R. Bearder, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, **1975**, **721**.
[3] J. R. Hanson and J. Hawker, *Phytochemistry*, 1973, **12**, 1073.
[4] J. R. Bearder, J. MacMillan, C. M. Wels, M. B. Chaffey, and B. O. Phinney, *Phytochemistry*, **1974**, **13**, **911**.
[5] J. R. Bearder, J. MacMillan, and B. O. Phinney, *Phytochemistry*, **1973**, **12**, **2655**.