Phytochemistry, 1973, Vol. 12, pp. 2351 to 2352. Pergamon Press. Printed in England.

INTERCONVERSION OF GIBBERELLIN A_1 TO GIBBERELLIN A_8 IN SEEDLINGS OF DWARF *ORYZA SATIVA*

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(*Received* 27 *February* 1973. *Accepted* 15 *May* 1973)

Key Word Index—*Oryza sativa*; Gramineae; dwarf rice; interconversion; gibberellins A_1 and A_8.

Abstract—[^{3}H]Gibberellin A_1 ([^{3}H]GA_1) applied to seedlings of dwarf rice (*Oryza sativa* L. cv. Tanginbozu) was metabolized to GA_8. Identification of GA_8 was made by gas–liquid radiochromatography using three liquid stationary phases.

RECENTLY, Nadeau and Rappaport[1] reported the interconversion of [^{3}H]GA_1 into GA_8 and GA_8-glucoside in seeds of *Phaseolus vulgaris*. Railton and Wareing[2] also studied the metabolism of [^{3}H]GA_1 in potato leaves and although the single acidic, radioactive conversion product they detected was not identified, its chromatographic and electrophoretic properties were consistent with those of GA_8. GA_4 has previously been shown to be converted to GA_1 in shoots of dwarf rice.[3] We now report the conversion of [^{3}H]GA_1 into GA_8 in shoots of the same species.

Tritium-labelled GA_1 (sp.act. 420 mCi/mmol) was applied (74 μCi total) in 95% ethanol to shoots of 25 dwarf rice plants. Twenty-four hr after application, shoots were excised from the seeds and roots, surface-washed in absolute methanol and ground in a mortar at 0° with 80% methanol and acid washed sand. The methanol was removed *in vacuo* at 35° and the residual aqueous phase adjusted to pH 9·0 by the addition of an equal volume of 0·5 M phosphate buffer. The buffer phase was washed 4 times at pH 9·0 with equal volumes of diethyl ether (total radioactivity $0{\cdot}08 \times 10^6$ cpm) and then adjusted to pH 3·0 and extracted 4 times with ethyl acetate ($17{\cdot}0 \times 10^6$ cpm) and 3 times with *n*-butanol ($0{\cdot}5 \times 10^6$ cpm). The residual buffer contained $0{\cdot}04 \times 10^6$ cpm. All counts were adjusted for quenching but not for counting efficiency.

The acidic, ethyl acetate fraction was chromatographed on a silica gel partition column[4,5] and eluted with increasing amounts of ethyl acetate in *n*-hexane. Fractions were combined according to counts obtained from direct scintillation spectrometry using Bray's scintillant.[6] The trimethylsilyl ethers of the methyl esters (TMSMe derivatives) of the combined fractions were analyzed by gas–liquid radiochromatography (GLRC) on 3 column liquid phases, 2% SE30, 2% QF1 and 1% XE60. Conditions for GLRC were identical to those described previously.[3,7] Combined fractions 19–22 contained one significant peak with retention times

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identical to those of TMSMe GA_8 standard (2% SE30, 25·4 min; 2% QF1, 17·4 min; 1% XE60, 17·5 min). Combined fractions 14–18 also contained a single radioactive peak, with identical retention times to those of TMSMe GA_1 standard (2% SE30, 15·4 min; 2% QF1, 14·0 min; 1% XE60, 15·3 min). Combined fractions 23 and 24 contained an unknown radioactive compound exhibiting long retention times on GLRC (2% SE30, 33·0 min; 2% QF1, 56·0 min; 1% XE60, 54·0 min).

The total radioactivity associated with each of the compounds was as follows: GA_8, $1·9 \times 10^6$ dpm; GA_1, $4·7 \times 10^7$ dpm; unknown compound, $2·4 \times 10^6$ dpm. The incorporation of GA_1 to GA_8 was 1·2% of the [^{3}H]GA_1 applied to the tissue.

The results reported here further confirm that GA_1 is preferentially hydroxylated at C-2 to produce GA_8 in higher plants and that such a conversion does occur in vegetative tissue.

Attempts were made to detect the presence of GA_8-glucoside in extracts of rice shoots. The *n*-butanol fraction was adjusted to neutral pH with saturated sodium bicarbonate solution, stored at −20° and subsequently reduced to dryness *in vacuo*. The residue was taken up in a minimum volume of distilled water and desalted on a column of charcoal (1·5 g)–celite (3 g) eluted with water. Radioactivity was removed from the column by washing with absolute methanol and derivatives of the residue from this fraction for GLRC were prepared as described previously.[3,7] Trace amounts of the free acid of GA_8 were present but the glucoside could not be detected. Recently, Nadeau and Rappaport (personal communication) have detected by co-chromatography using TLC the presence of [^{3}H]GA_8-glucoside after feeding [^{3}H]GA_1 to vegetative tissue of maize.

Acknowledgements—We gratefully acknowledge research support from NRC Grant A-2585 to R. P. Pharis and a N.A.T.O. Grant to J. MacMillan and R. P. Pharis. I. D. Railton was supported by a North Atlantic Treaty Organization–Science Research Council (London, U.K.) Postdoctoral Fellowship. We wish to thank Dr. L. C. Vining for supplying the [^{3}H]GA_1.