

SYNTHESIS OF THE LABELED GIBBERELLIN, GA<sub>9</sub>-17-<sup>14</sup>C

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## SUMMARY

GA<sub>9</sub>-17-<sup>14</sup>C was synthesized by introducing <sup>14</sup>C into GA<sub>9</sub>-17-norketone by the Wittig reaction. The NMR and mass spectra of free GA<sub>9</sub> and GA<sub>9</sub>-17-norketone are presented. The diphenylmethyl-<sup>14</sup>C-phosphine oxide is formed as a byproduct.

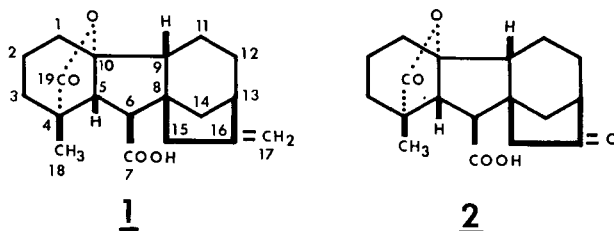
Keywords: GA<sub>9</sub>, GA<sub>9</sub>-17-norketone, NMR, mass spectra, Wittig reaction, diphenylmethylphosphine oxide.

## INTRODUCTION

The gibberellins (GAs) are diterpenoid acids with hormonal function and are essential for normal growth and development in higher plants. At the present time, the metabolism of GA<sub>9</sub> in Pisum sativum is under investigation by our research group. Radioactive GAs have been used in numerous studies of the metabolism of GAs (1,2,3). The introduction of <sup>14</sup>C into C-17 produces a precursor which retains its label throughout the metabolic conversions.

The preparation of radioactive GA<sub>9</sub> by the Wittig reaction has been previously described by Cross *et al.* (4) and by Bearder *et al.* (5). Cross *et al.* have used the GA<sub>9</sub>-17-norketone methyl ester in the Wittig reaction to prepare the GA<sub>9</sub> methyl ester, which then had to be hydrolyzed to obtain the free GA<sub>9</sub> (1). Bearder *et al.*, have used the GA<sub>9</sub>-17-norketone (2) in the Wittig reaction, but obtained impure radioactive GA<sub>9</sub>. In this communication we report a method for preparation of pure radioactive GA<sub>9</sub> from GA<sub>9</sub>-17-norketone without hydroly-

sis. The radioactive byproduct of the Wittig reaction is identified. NMR and mass spectra of free GA<sub>9</sub> and GA<sub>9</sub>-17-norketone are presented for the first time.



#### RESULTS AND DISCUSSION

For the preparation of GA<sub>9</sub>-17-<sup>14</sup>C, the Wittig reaction was carried out by the addition of GA<sub>9</sub>-17-norketone to the mixture of methyl-<sup>14</sup>C-triphenylphosphonium iodide in toluene and potassium *t*-butoxide in *t*-butyl alcohol. The Wittig reaction is often carried out in diethyl ether, but the GA<sub>9</sub>-17-norketone is insoluble in diethyl ether. Toluene was used in our method, because the GA<sub>9</sub>-17-norketone is soluble in toluene and there is no chance of contamination with peroxides. This method is not suitable for the preparation of GA<sub>9</sub>-17-<sup>3</sup>H<sub>2</sub>, because tritium will exchange with *t*-butyl alcohol after the ylide is formed (6). The Wittig reaction also produces the diphenylmethyl-<sup>14</sup>C-phosphine oxide, Ph<sub>2</sub><sup>14</sup>CH<sub>3</sub>PO. Its structure was determined by NMR and mass spectra (electron impact and chemical ionization)(7). This compound has not been previously reported as a byproduct of the Wittig reaction. It was reported that [Ph<sub>3</sub>PCH<sub>3</sub>]<sup>+</sup>[BPh<sub>4</sub>]<sup>-</sup> decomposes with concd. aq. KOH and gives Ph<sub>2</sub>CH<sub>3</sub>PO (8).

The proton NMR spectra of GA<sub>9</sub> and its norketone are presented in Fig. 1. The chemical shifts of some protons of the GA<sub>9</sub> methyl ester have been described by Hanson (9), but the spectrum of the GA<sub>9</sub> methyl ester was not given. The chemical shifts are identical in GA<sub>9</sub> and its norketone. Both spectra show the protons of the C-18 methyl group at δ 1.10 and a pair of doublets at δ 2.47 and 2.72, due to the C-5 and C-6 protons, with a coupling constant J = 10.3 cpm. The high coupling constant may be due to the

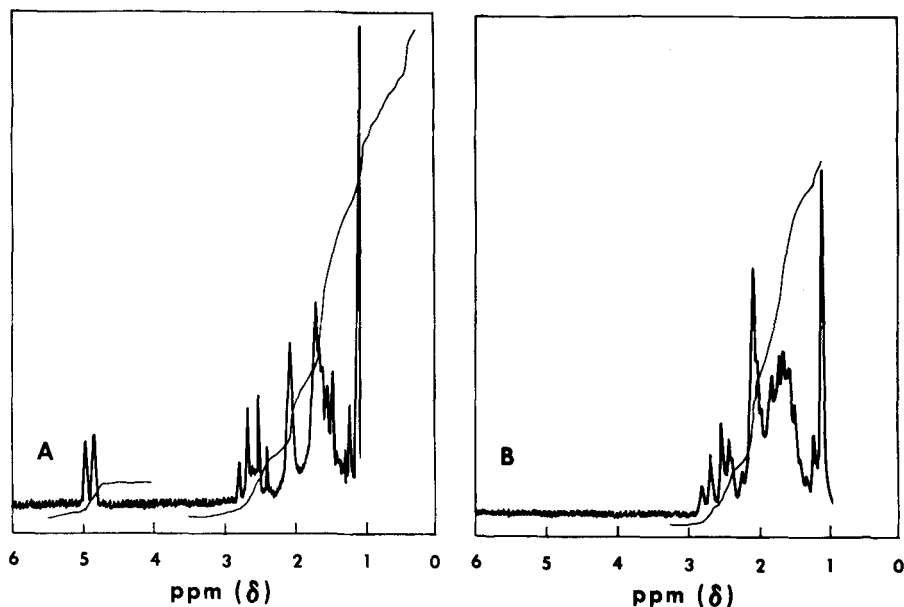


Fig. 1. Proton NMR spectra of  $GA_9$  (A) and  $GA_9-17$ -norketone (B).

two C-H bonds being nearly coplanar. The C-17 methylene protons,  $\delta$  4.83, and 4.96, are visible in the  $GA_9$  spectrum but not in the norketone spectrum. The spectrum of the norketone also shows the deshielding of protons on C-13 and C-15, but the exact chemical shifts of these protons cannot be clearly determined from this spectrum because of band overlap.

$^{13}C$ -NMR data of  $GA_9$  and  $GA_9-17$ -norketone are presented in Table 1. C-12 and C-14 of the norketone are shielded, while C-13, C-15, and C-16 are deshielded by the C-16 carbonyl group. The  $^{13}C$ -NMR data for  $GA_9$  have been published previously (10, 11) and our data on  $GA_9$  are identical. However, the  $^{13}C$ -NMR data for the  $GA_9-17$ -norketone have not been previously presented, although data for the  $GA_9-17$ -norketone methyl ester are published (11). The C-7 resonance of the  $GA_9-17$ -norketone moves upfield to 172.5 ppm upon formation of the methyl ester of the carboxylic acid (11).

TABLE 1  
 $^{13}\text{C}$  NMR data (p.p.m. from TMS, in  $\text{CDCl}_3$ )

Carbon	$\text{GA}_9$	$\text{GA}_9$ -17-Norketone
1	30.8	30.5
2	19.4	19.3
3	34.4	34.4
4	49.0	49.3
5	58.1	58.0
6	51.6*	52.9*
7	177.5*	176.2*
8	51.5	49.8
9	54.0	54.2
10	93.3	93.1
11	16.2	16.8
12	31.4	24.9
13	38.9	44.8
14	36.8	34.6
15	44.3	50.4
16	156.6	221.1
17	107.5	---
18	17.3	17.3
19	179.3	179.0

\*Broad peaks at repetition rates of 2.0 sec.

The mass spectra of  $\text{GA}_9$  and its norketone are presented in Fig. 2. The mass spectrum of  $\text{GA}_9$  methyl ester has been reported by Takahashi *et al.* (12) and Binks *et al.* (13). There are significant differences between the mass spectra of  $\text{GA}_9$  and  $\text{GA}_9$  methyl ester. The mass spectrum of  $\text{GA}_9$  methyl ester shows the prominent ions due to the methoxycarbonyl group, which is not very informative.

$\text{GA}_9$  exhibits prominent peaks due to M-17 (HO), M-18 ( $\text{H}_2\text{O}$ ), M-44 ( $\text{CO}_2$ ), M-46 ( $\text{CO}_2\text{H}_2$ ), M-59 ( $\text{CO}_2$ ,  $\text{CH}_3$ ), M-87 ( $\text{CO}_2$ ,  $\text{C}_3\text{H}_7$ ), M-89 ( $\text{CO}_2$ ,  $\text{CO}_2\text{H}$ ), M-112 ( $\text{CO}_2$ ,  $\text{C}_5\text{H}_8$ ), and M-113 ( $\text{CO}_2$ ,  $\text{C}_5\text{H}_9$ ). The fragment ions of M-17 (HO) and M-18 ( $\text{H}_2\text{O}$ ) are not visible in the mass spectrum of  $\text{GA}_9$  methyl ester (13). This suggests that these ions are due to the loss of HO from the carboxylic acid. The ions M-44 ( $\text{CO}_2$ ) and M-46 ( $\text{CO}_2\text{H}_2$ ) are not prominent in the  $\text{GA}_9$  methyl ester spectrum (13). The elimination may be mostly from the carboxylic acid. The ions M-87 ( $\text{CO}_2$ ,  $\text{C}_3\text{H}_7$ ), M-89 ( $\text{CO}_2$ ,  $\text{CO}_2\text{H}$ ), M-112 ( $\text{CO}_2$ ,  $\text{C}_5\text{H}_8$ ), and M-113 ( $\text{CO}_2$ ,  $\text{C}_5\text{H}_9$ ) may be due to the initial loss of  $\text{CO}_2$  of the lactone. The subsequent elimination of  $\text{C}_3\text{H}_7$ ,  $\text{C}_5\text{H}_8$ , and  $\text{C}_5\text{H}_9$  may be from Ring A. These ions were also shown in

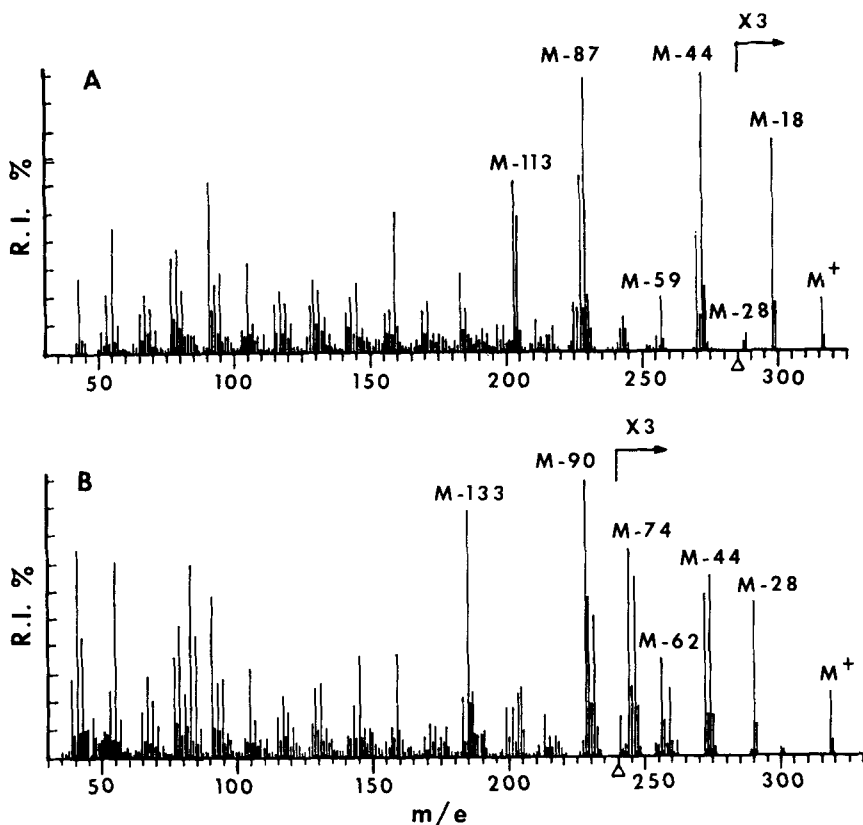


Fig. 2. Mass spectra of  $GA_9$  (A) and  $GA_9-17$ -norketone (B).

the  $GA_9$  methyl ester spectrum (13) without interpretation. The reactions are consistent with a study of metastable ions and the accurate mass of each ion. The measured mass of  $GA_9$  is 316.1673 and the calculated mass is 316.1674.

$GA_9-17$ -norketone exhibits prominent peaks due to M-28 (CO), M-44 ( $CO_2$ ), M-46 ( $CO_2H_2$ ), M-59 ( $CO_2$ ,  $CH_3$ ), M-62 ( $CO_2$ ,  $H_2O$ ), M-72 ( $CO_2$ , CO), M-74 ( $CO_2H_2$ , CO), M-90 ( $CO_2$ ,  $CO_2H_2$ ), and M-133 ( $CO_2$ ,  $CO_2H_2$ ,  $C_2H_3O$ ). The fragmentation of M-28 (CO) is not prominent in the spectrum of  $GA_9$ . This suggests that the elimination is mostly from the C-16 position. M-133 ( $CO_2$ ,  $CO_2H_2$ ,  $C_2H_3O$ ) may be due to the initial elimination of the  $CO_2$  of the lactone and then the elimination of  $CO_2H_2$  of the carboxylic acid and finally the elimination of  $C_2H_3O$  at the C-15 and C-16 positions. The measured mass of  $GA_9-17$ -norketone is 318.1463 and the calculated mass is 318.1467.

## EXPERIMENTAL

MethodsNuclear Magnetic Resonance (NMR)

Proton NMR spectra were obtained in  $\text{CDCl}_3$  at  $34^\circ\text{C}$  with tetramethylsilane (TMS) as an internal reference on a Varian EM-390 instrument (Varian/Instrument Division, Palo Alto, Ca., U.S.A.\*), operating at 90 MHz.  $^{13}\text{C}$ -NMR spectra were obtained in  $\text{CDCl}_3$  at  $24^\circ\text{C}$  with TMS as internal reference on a JEOL PFT-100 Fourier transform spectrometer (JEOL Ltd., Tokyo, Japan), operating at 25.03 MHz.  $^{13}\text{C}$ -NMR spectra were run with a flip angle of 48 degrees and a 2-sec. repetition rate.

Mass Spectrometry (MS)

Low-resolution mass spectra were obtained (E.I., 70 e.v., direct probe sample introduction), using a double-focusing magnetic-sector mass spectrometer, Model MM-70/70F (V.G. Micromass, Ltd., Altrincham, Ches., England). Exact masses of molecular ions were determined with a CEC 21-110A mass spectrometer (Du Pont Co. Instrument Products, Wilmington, Del., U.S.A.) by peak matching. The ion source temperature was  $200^\circ\text{C}$ . The metastable peaks were observed by B/E and  $\text{B}^2/\text{E}$  linked scans (14,15) on the MM - 70/70 F instrument.

Preparation of  $\text{GA}_9$ -17-norketone (ent-10 $\beta$ -hydroxy-17,20-dinorgibberell-16-oxo-7,19-dioic acid 19 $\rightarrow$ 10-lactone)

$\text{GA}_9$  (20 mg), dissolved in tetrahydrofuran (20 ml), was added to an aqueous solution (10 ml) of  $\text{OsO}_4$  (8 mg) and  $\text{NaIO}_4$  (500 mg). The mixture was stirred at room temperature for 2 days (16). Tetrahydrofuran was then removed by distillation, phosphate buffer (20 ml, 0.5 M, pH 3) was added, and the mixture was extracted with ethyl acetate.  $\text{GA}_9$ -17-norketone was isolated from the ethyl acetate extract by preparative thin-layer chromatography (TLC) on a

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\* Reference to a company or product does not imply approval or recommendation by the U.S. Department of Agriculture to the exclusion of others that might be suitable.

Silica Gel H layer, 20 x 20 cm, 0.5 mm thick, developed with methylene chloride - methanol - formic acid (50:50:1). The norketone band on the plate ( $R_f$  ca. 0.5) is easily located by inspection. This band was eluted with ethyl acetate, and the norketone was crystallized from ethyl acetate - hexane. The crystals formed a spheroidic aggregate of very fine needles (15 mg), m.p. 193-194°C. The m.p. was not previously reported.

Preparation of GA<sub>9</sub>-17-<sup>14</sup>C (ent-10β-hydroxy-20-norgibberell-16-ene-7,19-dioic acid 19→10 lactone)

Methyl-<sup>14</sup>C iodide (1 mCi, 6.8 mg, New England Nuclear, Boston, Mass., U.S.A.), was dissolved in benzene (2.5 ml) with slightly loss due to its volatile nature and was then added to triphenylphosphine (50 mg). The unlabelled methyl iodide (18.2 mg) was added to the mixture. After stirring the mixture at room temperature for 2 days, the methyl-<sup>14</sup>C-triphenylphosphonium iodide formed was collected by filtration and dried (62 mg) (4). Methyl-<sup>14</sup>C-triphenylphosphonium iodide was diluted to 75 mg with unlabelled methyltriphenylphosphonium iodide and was dissolved in 4 ml of dry toluene (dried with Molecular Sieves, Type 4A, Fisher Scientific Company, Fair Lawn, New Jersey) under nitrogen and a solution of potassium *t*-butoxide in *t*-butyl alcohol (0.3 ml, 0.61 N) was added (17). The resultant solution was yellow due to the ylide formation. The GA<sub>9</sub> 17-norketone (15 mg) (17), dissolved in dry toluene (1 ml), was added, and the mixture was stirred overnight at room temperature. After the addition of phosphate buffer (20 ml, 0.5 M, pH 3), the mixture was extracted with ethyl acetate.

The GA<sub>9</sub>-17-<sup>14</sup>C formed was purified by column chromatography on silica gel (50-200 mesh, Mallinckrodt, Inc., St. Louis, Mo., U.S.A.). The ethyl acetate extract is not soluble in hexane. It was dissolved in 5 ml of an ethyl acetate - hexane (4:6) mixture and applied to a chromatographic column (1 x 5 cm). GA<sub>9</sub>-17-<sup>14</sup>C was eluted by ethyl acetate-hexane (4:6) (10 ml). The byproduct, diphenylmethyl-<sup>14</sup>C-phosphine oxide in pure form, was then eluted by ethyl acetate - hexane (5:5)(10 ml). The GA<sub>9</sub>-17-<sup>14</sup>C fraction was dried, redissolved in 5 ml of ethyl acetate - hexane (2:8), and purified by a second

column chromatography. The unreacted GA<sub>9</sub>-17-norketone was eluted after GA<sub>9</sub>-17-<sup>14</sup>C. GA<sub>9</sub> (R<sub>f</sub> ca. 0.8), GA<sub>9</sub>-17-norketone (R<sub>f</sub> ca. 0.5), and diphenylmethylphosphine oxide (R<sub>f</sub> ca. 0.3) were located by TLC on a Silica Gel G layer, 0.25 mm thick, developed with methylene chloride - methanol - formic acid (50:50:1). Both the GA<sub>9</sub>-17-<sup>14</sup>C (10 mg, 4 mCi/mmol) and diphenylmethyl-<sup>14</sup>C-phosphine oxide (24 mg, 4 mCi/mmol) obtained gave a single peak when the TLC plate was scanned (Radiochromatogram Scanner, Model 7200, Packard Instrument Co., Downers Grove, Ill., U.S.A.).

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