Chemistry of Natural Compounds, Vol. 29, No. 4, 1993

1α,2α-EPOXYGIBBERELLIN A_3: PARTIAL SYNTHESIS, NMR SPECTRA, BIOLOGICAL ACTIVITY, AND CRYSTAL STRUCTURE OF ITS METHYL ESTER

N. A. Pankrushina, A. G. Druganov, I. Yu. Bagryanskaya, Yu. V. Gatilov, V. A. Raldugin, M. M. Shakirov, E. V. Evtushenko, and V. A. Saprykin

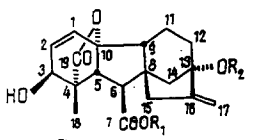
UDC 547.472.3−548.737−577.151.1

1α,2α-Epoxygibberellin A_3 has been obtained for the first time in almost quantitative yield by the dehydroiodination of the known 1β-iodogibberellin A_{56}, and the structure of its molecule has been established by the XSA method. An interpretation of all the signals in its ^1H and ^{13}C spectra has been made from the results of two-dimensional NMR. In the amylase biotest, 1α,2α-epoxgibberellin A_3 exhibited an activity amounting to one third of the activity of gibberellin A_3 at concentrations of 10^{-7} to 10^{-9} M.

1,2-Epoxygibberellins may be the starting compounds for the synthesis of new gibberellin derivatives; moreover, it is not known how the biological activity of the phytohormone molecule is affected by the replacement of a Δ^1-double bond by an epoxide group.

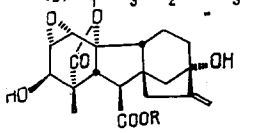
The epoxidation of gibberellin A_3 (GA_3) (I) or its methyl ester with peracids takes place at the sterically unhindered exomethylene group [1], and the selective introduction of an epoxy group into the 1-2 position of the molecule is known only for the methyl esters of 13-O-acetates on the use of a modified Sharpless reagent. In this reaction, the methyl esters of 13-O-acetyl-GA_3 (II) and its 3-epimer gave the expected epoxides of (III) and of 1,2,3-tri-epi-(III), respectively [2].

We have found that an epoxide with the transposition of the epoxide and hydroxy groups — 1α,2α-epoxygibberellin A_3 (IV) — can be obtained without the use of protective groups and in almost quantitative yield on the dehydroiodination of the known iodolactone (V), obtained in two stages from GA_3 [3]. The reaction takes place in acetonitrile in the presence of potassium carbonate and a crown ether, with boiling for 5 h (monitoring by HPLC).



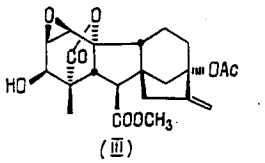
(I) $R_1=R_2=H$

(II) $R_1=CH_3$; $R_2=OCCH_3$

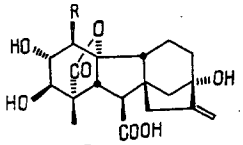


(IV) R=H

(VII) $R=CH_3$



(III)



(V) R=J

(VI) R=H

It has also been established that the epoxide (IV) is formed from the iodolactone (V) when the latter is treated with sodium tetrahydroborate in DMSO. Takahashi et al. [3] attempted to obtain gibberellin A_{56} (VI) in this way but did not achieve a satisfactory result, and the reaction mixture was not investigated.

The structure and stereochemistry of the epoxide (IV) molecule were established by the x-ray structural analysis of its methyl ester (VII) and are shown in Fig. 1, which also gives the lengths of the most extended bonds. The conformations of the rings of the (VII) molecule are the same as those for the GA_3 molecule [4].

Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences. Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 549-553, July-August, 1993. Original article submitted February 9, 1993.

0009-3130/93/2904-0479$12.50

TABLE 1. Details of the ^{13}C and ^{1}H NMR Spectra of Epoxide (IV) (in pyridine-d_5, c = 0.02 and 0.002 M, respectively)

i	$\delta(C_i)$	$\delta(H_i)$
		2,56 br.d (3,5 Hz)
1	53,74 d	2,62 dd (3,5 and 0,9 Hz)
2	54,01 d	4,43 br.s ($W_{1/2}$ = 2,5 Гц)
3	69,64 d	—
4	50,26 s	3,77 d (10,6 Hz)
5	52,94 d	3,25 d (10,6 Hz)
6	51,45 d	—
7	174,64 s	—
8	50,00 s	2,25 d d (11,0 and 5,8 Hz)
9	52,20 d	—
10	88,15 s	1,79 m; 2,19 m
11	17,47 t	1,99 m; 2,37 ddd (12,0, 8,5 and 2,0 Hz)
12	39,79 t	—
13	77,58 s	2,31 br.s (2H-14)
14	45,58 t	
15	43,74 t	2,50 br.d (15,5 Hz) 2,81 ddd (15,5, 3,0, 3,0 Hz)
16	159,00 s	—
17	106,75 t	5,04 nar.m; 5,60 nar.m
18	15,51 q	1,63 s (3H)
19	178,26 s	—

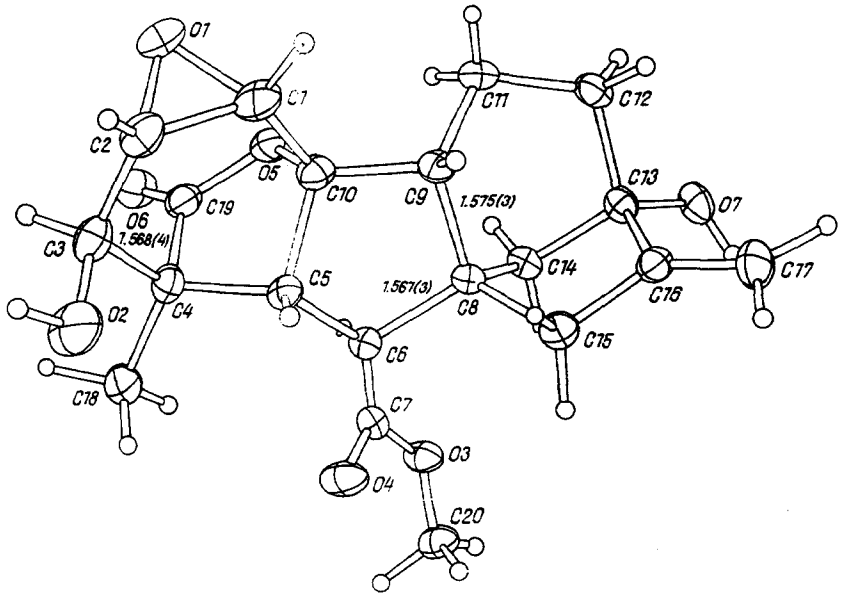


Fig. 1. Spatial structure of the (VII) molecule.

The interpretation of the NMR spectra of epoxide (IV) (Table 1) was carried out with the use of two-dimensional ^{13}C-^{1}H NMR spectroscopy (COSY, COLOC). The ^{13}C NMR spectrum was very similar to that of GA_3 [5] and differed appreciably from it only by the magnitudes of the chemical shifts of the signals due to the C-10 and C-1 to C-4 carbon atoms. In the PMR spectrum of epoxide (IV), as in the spectrum of GA_3 [5], the signals of the geminal protons at C-14 practically coincided. We may also mention the small value of the $J_{2,3}$ SSCC (0.9 Hz) and the presence of long-range spin-spin coupling between the H-1 and H-3 protons, appearing in a broadening of the components of the H-1 signal, which decreased on double resonance with suppression of the H-3 proton.

The methyl ester of 1α,2α-epoxygibberellin A_3 (VII) is soluble in $CDCl_3$, and in its PMR spectrum (see the Experimental part), as in the spectrum of the acid (IV), the signals of the H-1 and H-2 protons appear in the form of a doublet ($J_{1,2}$ = 3.4 Hz) and a doublet of doublets ($J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 0.9 Hz), respectively. It seemed that the assignment of the signals for the H-1 and H-2 protons from the observed splittings was completely reliable, but when the PMR spectrum was recorded with contraction of the lines it was found that the components of the H-1 signal underwent additional splitting into

TABLE 2. Amylolytic Activities of the Gibberellin and Their Derivatives

Substance	Concentration, M			
	10^{-9}	10^{-8}	10^{-7}	10^{-6}
GA_3 (I)	100±7,0	231±3,7	300±17	415±9,3
GA_{56} (VI)	15,4±0,5	41,5±1,6	56,9±4,1	115±3,8
(V)	13,8±2,0	41,5±4	90,8±7,9	154±13,6
(IV)	27,7±2,0	75,4±8,4	115±3,7	217±17,4

*The activity values are given relative to the activity of GA_3 at c = 10^{-9} M, taken as 100.

TABLE 3

Atom	x	y	z	U	Atom	x	y	z	U
C1	6682(4)	3858(4)	4817(1)	51	C2	7303(5)	5090(4)	5118(1)	59
C3	7350(4)	6507(3)	4821(1)	51	C4	6856(3)	6450(3)	4163(1)	38
C5	5372(3)	5545(3)	4135(1)	31	C6	4535(3)	5444(2)	3540(1)	28
C7	3310(3)	6555(2)	3453(1)	29	C8	3894(3)	3914(2)	3514(1)	27
C9	4933(3)	3057(3)	3954(1)	34	C10	6095(3)	4093(3)	4193(1)	34
C11	5616(3)	1719(3)	3689(1)	46	C12	4374(3)	874(3)	3362(1)	40
C13	3175(3)	1812(3)	3048(1)	35	C14	3930(3)	3238(3)	2911(1)	33
C15	2154(3)	3712(3)	3675(1)	36	C16	1827(3)	2246(3)	3448(1)	35
C17	571(4)	1477(4)	3566(2)	52	C18	6759(4)	7931(3)	3911(2)	50
C19	7973(3)	5533(3)	3824(1)	40	C20	1692(4)	7635(3)	2755(2)	44
O1	8343(3)	4012(3)	4901(1)	69	O2	6288(4)	7406(3)	5102(1)	71
O3	2867(2)	6617(2)	2897(1)	38	O4	2791(2)	7311(2)	3821(1)	46
O5	7464(2)	4192(2)	3811(1)	40	O6	9146(2)	5889(3)	3563(1)	59
O7	2668(3)	1040(2)	2550(1)	47					

doublets (0.6 Hz). When the signal of the H-3 proton (narrow multiplet) was suppressed, the signals for the H-1 and H-2 protons acquired the form of unresolved doublets (AB system, $J_{A,B}$ = 3.4 Hz), which enabled the 0.6 Hz splitting to be interpreted as the $^4J_{1,3}$ SSCC. However, the closeness of the values of $J_{2,3}$ and $J_{1,3}$ makes the opposite assignment of the signals for H-1 and H-2 probable, as well. The correctness of the interpretation made was confirmed by the PMR spectrum recorded with the suppression of the H-3 signal followed by contraction of the lines. The components of the doublet H-2 signal in the spectrum were considerably narrower than those for H-1 ($W_{1/2}$ = 0.3 and 0.7 Hz, respectively). The broadening of the H-1 signal showed the presence of at least one more long-range spin-spin interaction between the four σ bonds (with H-5 or H-9). Such an interaction is impossible for the H-2 proton, which is reflected in the narrow form of the components of its doublet signal. It must also be mentioned that the cross peaks in the COLOC spectrum for a solution in Py-d_5 (C-4/H-1, C-10/H-2, C-3/H-1, H-2) cannot be used for an independent assignment of the H-1 and H-2 signals, since the cross peaks for C-1 and C-10 may also be given the opposite interpretations (C-4/H-2, C-10/H-1).

One of the methods of determining the specific biological activities of the gibberellins is the amylase test, which is based on the quantitative determination of the induction of the enzyme α-amylase under the action of the substance under test in aqueous solution on aleuronic cells of barley grains [6]. We investigated the epoxide (IV) in this test and also the iodolactone (V), using GA_3 and GA_{56} as controls. The results, which are given in Table 2, show that both the substances tested (IV) and (V) possess a pronounced gibberellin-like action, with the activity of the epoxide (IV) being twice that of the iodolactone (V) while the activity of the latter did not differ appreciably from that of GA_{56}.

EXPERIMENTAL

NMR spectra were recorded on a Bruker AM-400 instrument (400.13 MHz for ^1H and 100.614 MHz for ^{13}C), δ scale. The CSs are given relative to the residual signals of the solvents — pyridine-d_5 (7.19 ppm for ^1H and 143.05 ppm for ^{13}C) and $CDCl_3$ (7.24 ppm for ^1H). In the recording of the two-dimensional spectra we used standard pulse sequences.

The mass spectrum was recorded on a Finnigan MAT 8200 instrument (110°C, direct insertion), and the IR spectrum in KBr on a UR-20 instrument. The melting point was determined on Kofler stage, and optical rotation was measured on a

Polamat A polarimeter for a solution in CH_3OH. HPLC was conducted on an Ob'-4 microcolumn liquid chromatograph [7] using a 2 × 62 mm column containing the sorbent Nucleosil 5C-18 (FRG), the eluents being CH_3CN + 0.05 M CH_3COOH (15:85) (eluent A) and CH_3OH + 0.05 M CH_3COOH (35:65) (eluent B) at a rate of flow of 100 μl/min.

The iodolactone (V) (1β-iodogibberellin A_{56}) was obtained from GA_3 by the procedure of Takahashi et al. [3].

ent-1β,2β-Epoxy-3α,10,13-trihydroxy-20-norgibberell-16-ene 7,19-Dioic Acid 19,10-Lactone (IV). A solution of 1.00 g (2.05 mmole) of the iodolactone (V) in 120 ml of CH_3CN was treated with 0.87 g (6.3 mmole) of K_2CO_3 and 1.00 g of a complex of 18-crown-6 with CH_3CN. The reaction mixture was stirred with heating under reflux until the initial substance had disappeared completely from it (5 h, monitoring by HPLC, eluent A). After this, it was cooled, filtered, and evaporated to dryness. The residue was dissolved in 10 ml of water, the solution was acidified to pH 3 with hydrochloric acid, and the product was extracted with ethyl acetate (6 × 40 ml). The combined ethyl acetate extract was washed with aqueous sodium chloride and was dried with magnesium sulfate. After the solvent had been eliminated, the residue was dissolved in the minimum volume of acetone; hexane was added and filtration gave 0.73 g of the epoxide (IV) in the form of a white amorphous powder with $[\alpha]_{580}^{22}$ + 35.9° (c 0.39). IR spectrum: 1720 (COOH), 1785 (γ-lactone), cm^{-1}. For details of the ^1H and ^{13}C NMR spectra, see Table 1. Mass spectrum (m/z, %): 362.1324 (calculated for $C_{19}H_{22}O_7$ — 362.1365) (94), 344 (59), 284 (30), 231 (100).

The methyl ester (VII) was obtained by the addition of an excess of an ethereal solution of CH_2N_2 to a solution of the epoxide (IV) in CH_3OH. After the elimination of the solvent and crystallization of the products from a mixture of hexane and acetone, crystals were obtained with mp 186-187°C and $[\alpha]_{580}^{22}$ + 44.4° (c 0.27), which were used for XSA. PMR spectrum ($CDCl_3$, c = 0.04 M): 1.12 (3H, s, 3H-18), 2.70 (1H, d, J = 11.0 Hz, H-6), 3.11 (1H, d, J = 11.0 Hz, H-5), 3.20 (1H, br.d, H-1, $J_{1,2}$ = 3.4 Hz), 3.30 (1H, dd, H-2, $J_{2.1}$ = 3.4 Hz, $J_{2.3}$ = 0.9 Hz), 3.69 (3H, s, $COOCH_3$), 4.03 (1H, narrow m, $W_{1/2}$ = 2.5 Hz, H-3), 4.96 (1H, dd, J = 3.1 and 1.9 Hz, H-17a), and 5.27 (1H, m, H-17b) ppm.

Preparation of the Epoxide (IV) by the Interaction of Iodolactone (V) with $NaBH_4$. A solution of 0.50 g of (IV) in 20 ml of dried DMSO was treated with 0.5 g of $NaBH_4$, and the mixture was kept at 80-90°C until the initial substance had disappeared (70 min, monitoring by HPLC, eluent B). The cooled reaction mixture was treated with 8 ml of 0.1 N HCl, and the product was extracted with ethyl acetate (7 × 40 ml). The combined ethyl acetate extract was washed with water and with sodium chloride solution and, after drying and the elimination of the solvent, an amorphous product was obtained which was purified by precipitation with hexane from acetone solution. This gave 0.17 g of the epoxide (IV), identical in its spectral characteristics with the specimen described above.

X-Ray Structural Experiment with the Methyl Ester (VII). This was performed on a Syntex $P2_1$ diffractometer (Cu-K_α radiation with a graphite monochromator, $\theta/2\theta$ scanning). Crystallographic characteristics: a = 8.5528 (9), b = 9.548 (1), c = 22.930 (3) Å, V = 1872.5 (4) $Å^3$; space group $P2_12_12_1$, $C_{20}H_{24}O_7$, Z = 4, d_{calc} = 1.33 g/cm^3, $2\theta_{max}$ = 140°; number of reflections — 1746, R = 0.041, R_w = 0.042, S = 0.4. The structure was interpreted by the direct method (SHELX 86 program) and was refined by an anisotropic-isotropic (for the H atoms) full-matrix approximation (SHELX 76 program). The atomic coordinates obtained are given in Table 3.

Biotesting. Inembryonate halves of ripe barley seeds (*Hordeum* L. var. Himalaya) were sterilized by steeping in a 0.5% aqueous solution of NaOCl for 45 min. After this, they were washed repeatedly with water (here and below, sterile distilled water was used) and were left in water at +2°C. After 20 h, the grain halves were placed in sterile bottles and were covered with a sterile aqueous solution containing $CaCl_2$ (c = 20 mM) and the substance under test (c = 10^{-6}-10^{-9} M). The volume of this solution was 1 ml for three half-grains of barley. Incubation was carried out at +30°C for 48 h. After this, the bottle were placed in a refrigerator (−20°C) and were kept for the determination of amylolytic activity by Gibson's method [6]. The results obtained, normalized to the index for GA_3 at c = 10^{-9} M are given in Table 2.

REFERENCES

1. K. Schreiber, G. Schneider, and G. Sembdner, Tetrahedron, 1437-1444 (1966).
2. Z. Wang and W. Zhou, Synth. Commun., **19**, 2627-2632 (1989).
3. N. Murofushi, M. Sugimoto, K. Itoh, and N. Takahashi, Agric. Biol. Chem., **43**, 2179-2185 (1979).
4. L. Kutschabsky and G. Adam, J. Chem. Soc., Perkin Trans. I, 1653-1655 (1983).
5. A. Preiss, G. Adam, D. Saman, and M. Budesinsky, Magn. Reson. Chem., **25**, 239-243 (1987).

6. R. A. Gibson, J. Cell. Sci., **22**, 413-425 (1971).
7. G. I. Baram, M. A. Grachev, N. A. Komarova, M. P. Perelroyzen, Yu. A. Bolvanov, S. V. Kuzmin, V. V. Kargaltsev, and E. A. Kaper, J. Chromatogr., **264**, 69 (1983).