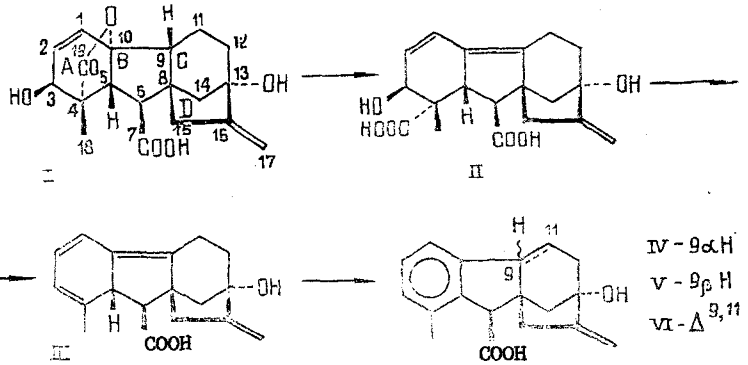
PROOF OF FORMATION OF AN UNSTABLE CONJUGATED TRIENE ON DECOMPOSITION OF GIBBERELLIN A_3 IN WATER

N. A. Pankrushina, A. V. Tkachev,
and A. G. Druganov

UDC 547.992+543.544+577.17

It has been shown by the methods of ^1H NMR and high-performance liquid chromatography with recording of UV spectra that in aqueous solutions of gibberellin A_3, via gibberellenic acid, an unstable conjugated triene having λ_{max} 326 nm is formed which is readily converted into allogibberic acid and is isolated as a mixture (1:2) with this acid. In the ^1H NMR spectrum of the triene, confirming its structure, spin-spin coupling both of the H-5 proton and of the protons of the methyl group with all the H-1, H-2, and the H-3 protons is observed. The precursor of allogibberic acid has been ascribed the structure of (−)-13-hydroxy-19,20-dinorgibberella-1,3,9,16-tetraen-7-oic acid with a conjugated 1,3,9-trienic system.

The chemical properties of the highly active phytohormone gibberelin A_3(I) and the products of its transformation are an object of intensive study [1]. It is known that in aqueous solutions gibberellin A_3 (gibberellic or gibberelloic acid) (I) forms the dicarboxylic gibberellenic acid (GEA) (II), which is also unstable and, on decomposing, gives a mixture of monocarboxylic aromatic acids consisting of allogibberic acid (AGA) (IV), 9-epiallogibberic acid (9-epi-AGA) (V), and dehydroallogibberic acid ($\Delta^{9,11}$-AGA) (VI) [2-4]. Pryce has put forward the hypothesis that an intermediate compound in the course of the decomopsition of GEA (II) is the unstable conjugated triene (III), from which the monocarboxylic aromatic acids (IV), (V), and (VI) are also formed. The basis for this hypothesis was the appearance in the UV spectrum of an aqueous solution of GEA (II) after heating of an absorption band with λ_{max} 326 nm and the disappearance of this band when air was passed through the solution or it was heated further, with the formation of a mixture of the acids (IV), (V), and (VI) [4]. The aim of the present work was to check the hypothesis of the appearance of the triene (III) in the course of the transformation of gibberellin A_3 in aqueous solution. To study the transformation of gibberellin compounds in aqueous solutions we made use of the method of microcolumn reversed-phased high-performance liquid chromatography (MC RP-GPLC) with two-wave detection [5], and chromatographed the aqueous solutions without any treatment whatever.



When the mixtures of products of the decomposition of aqueous solutions both of gibberellin A_3 (I) and of GEA (II) were analyzed by MC RP-HPLC we did, in fact, observe a new substance, traditionally called substance T, with a chromophoric group unusual for gibberellin

---

Novosibirsk Institute of Organic Chemistry, Siberian Branch, USSR Academy of Sciences. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 738-743, September-October, 1988. Original article submitted May 14, 1987; revision submitted April 26, 1988.

0009-3130/88/2405-0630$12.50

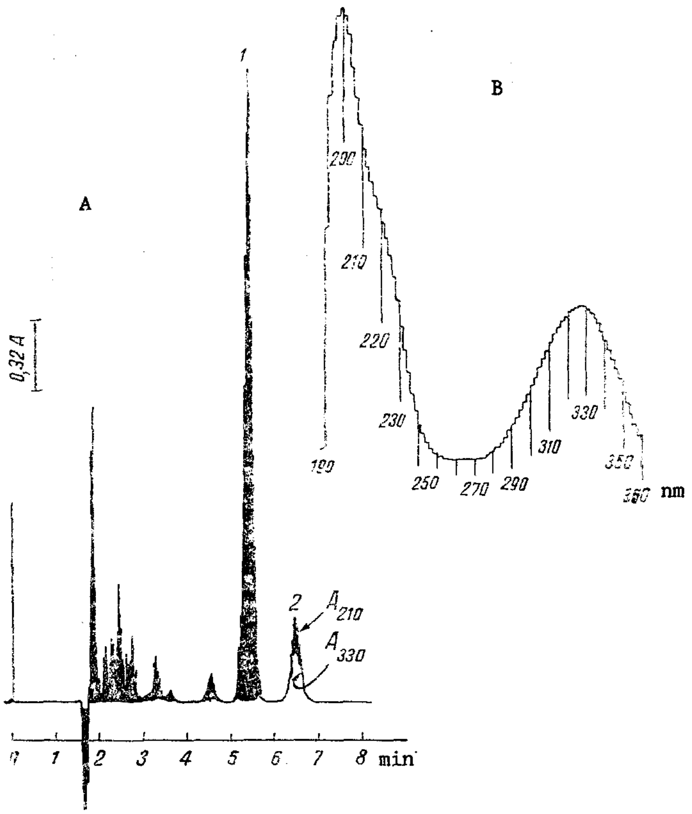


Fig. 1. A. Reversed-phased chromatography of a mixture of AGA (IV) and of the triene (III) (substance I) — peaks 1 and 2, respectively. The upper envelope of the hatched peaks corresponds to absorption at 210 nm and the lower envelope to that at 330 nm. B. Quasismooth UV spectrum of peak 2 (the conditions of analyzing and recording the UV spectrum are given in the Experimental part).

(λ_{max} 326 nm) and established that this compound is formed from GEA (II) and is unstable and is very readily transformed into AGA (IV). It was found that on chromatography, in contrast to the initial A_3 (I) and GEA (II), which are eluted in analyses by 35% methanol in water, substance T was eluted from the column with 70% methanol in water, and in an alkaline eluent (0.02 M Tris-acetate, pH 8.0) substance T and AGA (IV) were not separated, although complete separation was achieved in an acid eluent (Fig. 1). Such closeness of the chromatographic properties of these two related substances is possible only if they contain the same numbers of polar substituents — carboxy and hydroxy groups.

We have succeeded in obtaining this intermediately formed substance T in a mixture* with AGA in a ratio of 1:2, according to the PMR spectrum. Because of the instability of substance T, to determine its structure we studied the PMR spectrum of the mixture obtained (Table 1). For the new component of the mixture it was possible to observe in the PMR spectrum signals of the following protons: three olefinic protons of a conjugated system of double bonds at δ 6.16, 5.87, and 5.67 ppm, two protons of an exomethylene group at δ 5.18 and 5.00 ppm, and two protons at δ 3.92 and 3.04 ppm. The signal of the protons of the methyl group of substance T formed a broadened singlet (width of the signal at half-height 6 Hz) at δ 1.75 ppm, and the position of this signal indicated that the methyl group was attached to a carbon atom at a double bond. The hydrogen atoms of the methyl group were linked by spin-spin coupling (SSC) with all three protons of the conjugated olefinic system,

---

*One spot in TLC analysis.

TABLE 1. Parameters of the PMR Spectrum of Allogibberic Acid (IV) and of the Triene (III)*

i	δH_i, ppm			
	IV**	III**	IV***	III***
1	6,98 d*	6,31 d ($W_{1/2}=5$)	6,95 d*	6,16 d ($W_{1/2}=4$)
2	7,16 t	5,91 dd	7,17 t	5,87 dd
3	7,01 d*	5,68 m ($W_{1/2}=12$)	7,04 d*	5,67 m ($W_{1/2}=11$)
5	—	4,44 m ($W_{1/2}=24$)	—	3,9 m
6	4,13 s	3,27 d	3,99 s	3,04 d
9	2,6—3,0	—	2,84 dd	—
17a, b	4,73 s ($W_{1/2}=6$)	4,98 s ($W_{1/2}=5$)	4,77 d	5,00 t
	5,31 s ($W_{1/2}=8$)	5,47 s ($W_{1/2}=6$)	5,01 t	5,18 t
18	2,34 s	1,91 s ($W_{1/2}=6$)	2,22 s	1,75 s ($W_{1/2}=6$)

*200.13 MHz; the chemical shifts marked with asterisks should, possibly, change places within the limits of a column; $W_{1/2}$ is the width of the signal at half-height (Hz) for signals of undetermined multiplicity or the width of the component for well-resolved multiplets; T = 295 K; accuracy of the determination of the SSC constants ±0.2 Hz.
†c = 3% in pyridine-d_5; δH (COO$\underline{H}$, RO$\underline{H}$) 6.5 ppm ($W_{1/2}$ = 10); SSC constants (J, Hz); AGA (IV): 1,2 = 8.0; 2,3 = 8.0; the triene (III): 1,2 = 10.0; 2,3 = 6.0; 5,6 = 11.0.
‡c = 0.7% in deuterochloroform; δH (COO$\underline{H}$, RO$\underline{H}$) 5.2 ppm ($W_{1/2}$ = 150); SSC constants (J, Hz); AGA (IV); 1,2 = 8.0;

2.3 = 8.0; 9.1αβ = 6.0 and 12.0; 15αβ, 17ab = 2.5; the triene (III): 1.2 = 9.5; 1.5 = 2.5; 1.3 ≠ 0; 2.3 = 6.0; 2.5 = 2.0; 3.5 = 3.0; 5.6 = 10.5; 15αβ, 17ab = 2.5.

as was readily observed with the aid of homonuclear double resonance.* All three olefinic protons of the conjugated system were linked successively with one another by SSC. The facts presented permit the conclusion that in the molecule of substance T, ring A has the structure of 19,20-dinorgibberella-1,3-diene.

In the spectrum of substance T, a broad signal was observed at δ 3.92 ppm which was linked by small SSC constants (2-3 Hz) with the H1, H2, and H3 atoms. Such a signal can be ascribed to a proton present in the allyl position of a dienic system. Furthermore, the size of the chemical shift of this signal permits the conclusion that the corresponding proton occupies a twofold allyl position. In the spectrum of substance T, a signal is observed at δ 3.04 ppm which is linked by a SSC constant only with the signal at δ 3.92 ppm. These signals can be ascribed to H-6 and H-5 protons, respectively, in the structure of 19, 20-dinorgibberella-1,3,9-triene, but not to H-9 and H-10 protons in the alternative gibberella-1,3,5-triene structure.

In the process of the chromatographic analysis of the mixture of AGA (IV) and substance T, for the homogeneous peak of this substance (Fig. 1, a, peak 2) the UV spectrum was recorded (Fig. 1, b); its long-wave part contained a single absorption band with λ_{max} 326 nm, which confirmed the conjugation of a 1,3-diene with another double bond in the 9, 10-position. Practically the only literature example of a compound with an analogous conjugated trienic system was found to be 1,2,3,4,6-pentamethyl-5-methylenecyclohexa-1,3-diene, the UV spectrum of which also has only one absorption band, with λ_{max} 307 nm [6].

---

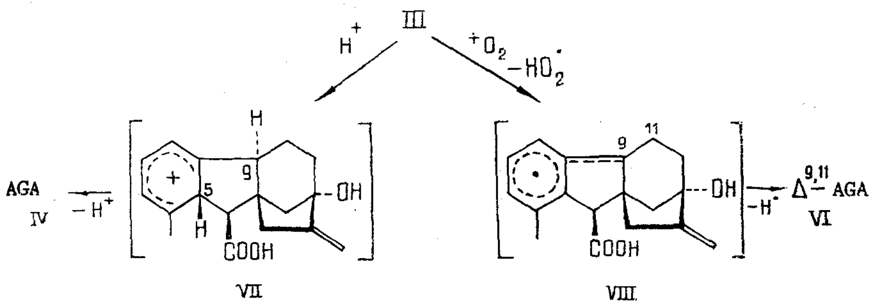
*When the sample was irradiated at the resonance frequency of the absorption of the protons of the methyl group, contraction of the signals of the protons of the conjugated olefinic system was due just to the disappearance of an interaction with the methyl group and not with other allylic protons, such as the H-11α and β atoms, the signals of which could fall into the 1.65-1.85 ppm region, i.e., close to the signal of the methyl group. It was shown by special experiments (double resonance at the frequency of the H-5 signal) that the signals of the allylic protons H-11β and H-11α were located in the 2.40-2.75 ppm region.

The β-orientation of the H-5 proton of substance T was determined from the known β-orientation of the H-9 of 9-epi-AGA (V), which is formed quantitatively from GEA (II) in aqueous solution simply on UV irradiation with simultaneous boiling, without the participation of the protons of the medium [7]. It follows from what has been said above that substance T participates in this photochemical transformation, and in it there is a [1—3]-sigmatropic migration of the H-5 hydrogen atom into position 9 which, as is known, takes place only by the suprafacial route [8].

Thus, the substance T that we have investigated is, in fact, the conjugated triene (III) and has a structure (−)-13-hydroxy-19,20-dinorgibberella-1,3,9,16-tetraen-7-oic acid.

It may be assumed, therefore, that when GEA (II) is heated in water a concerted reaction of decarboxylation and dehydration takes place in which the groups present on the neighboring C-3 and C-4 atoms of this acid (II) participate, with the formation of a new 3,4-double bond. Such a reaction has been described for β-hydroxy carboxylic acids. It occurs at a temperature of about 100°C if the β-hydroxy group is allylic [9], as is the case for GEA (II). The analogous decomopsition of alicylic β-hydroxy carboxylic acids requires more severe conditions [10].

Compound (III) with a conjugated trienic system possesses a high tendency for ring A to undergo aromatization, which accordingly leads to a high reactivity with electrophilic and radical particles. The electrophilic mechanism of the reaction of triene (III) can explain the ready formation of AGA (IV) on the decomposition of gibberellin A_3 (I) and GEA (II) in aqueous solutions: the attack of a proton on position 9 of triene (III) forms the arenonium cation (VII), which is stabilized in the usual way with the ejection of the H-5 proton and the formation of AGA (IV) having a less strained conformation of ring C than in the initial gibberellin A_3. In actual fact, when A_3 (I) was kept in 1 N hydrochloric acid for four days, AGA (IV) was obtained with a yield of 66% [11]. The reaction of the triene (III) with oxygen or other active radical particles will begin with the splitting out of the doubly allylic H-5 atom, leading to the formation of a less active radical of the benzyl type (VIII), changing, in the course of further transformations, into $\Delta^{9,11}$-AGA (VI), which has been detected in aqueous solutions containing A_3 (I) and GEA (II) [3, 4].



It is interesting to note that the antiradiation activity of gibberellin A_3 observed in plants [12] may be connected with the formation from it in the plant cell of the triene (III) and its interaction with the active radical particles appearing under the action of radiation.

Thus, in the present work we have shown the formation of triene (III) — yet another intermediate product in the complex process of the deactivation of the phytohormone gibberellin A_3 in aqueous solutions.

EXPERIMENTAL

Gibberellin A_3 (I) was obtained in the pure form by the crystallization from aqueous methanol [13] of "Gibberellin krystallicheskii" ["crystalline gibberellin"] produced by the "Sintez" combine, Kurgan. Gibberellenic acid (II) and allogibberic acid (IV) were synthesized by known procedures [11, 14].

Analysis was performed on an OB'-4 microcolumn liquid chromatograph, with a 2 × 62 mm column containing the sorbent Nucleosil 5C-18 (FRG) at a rate of flow of 100 μl/min. Detection was carried out at two wavelengths — 210 and 260 or 330 nm. UV spectra of the chromatographic peaks were recorded after flow had been stopped, the values of the optical density being recorded every 2 min.

PMR spectra (Table 1) were recorded on a Bruker WP 200 SY instrument.

Production of the Triene (III) from Gibberellin A_3 (I). Helium was passed through 5 ml of water for 20 min, and then 6 mg of A_3 was added. The reaction mixture was kept in an oil bath (130°C) with a reflux condenser for 25 min, with the continued passage of helium. An aliquot of the reaction mixture, without additional treatment was analyzed by the MC RP-HPLC method in a gradient of 800 μl of 30% methanol + 200 μl 50% methanol + 1200 μl of 70% methanol in a 0.05 M aqueous solution of formic acid, 8 μl of the mixture to be analyzed being deposited on the column. On the chromatogram, residual A_3 (I), GEA (II), and the triene (III) were identified from their retention times, by the method of adding known compounds, and from their UV spectra. With an increase in the time of heating to 60 min, the amount of the triene (III) in the reaction mixture did not change.

Formation of the Triene (III) from Gibberellenic Acid (II). Argon freed from oxygen, or helium, was passed through 30 ml of water for 20 min, and then 98 mg of GEA (II) was added and the mixture was kept in an oil bath (125°C) in a current of argon freed from oxygen or of helium, with reflux condenser, for 25 min. After cooling, the reaction mixture was filtered from the crystals that had deposited. The weight of the crystals after being dried in vacuum over P_2O_5 was 45 mg. A methanolic solution of the crystals (3 mg/ml) was analyzed by the MC RP-HPLC method (Fig. 1) with elution by 70% methanol in a 0.05 M aqueous solution of formic acid, 0.7 μl of solution being deposited on the column.

SUMMARY

It has been shown by the methods of PMR and MC RP-HPLC with the recording of UV spectra that, in water, gibberellin A_3 is converted via gibberellenic acid into (−)-13-hydroxy-19, 20-dinorgibberella-1,3,9,16-tetraen-7-oic acid, with a conjugated 1,3,9-trienic system, which is readily aromatized under the action of acids or atmospheric oxygen with the formation of allogibberic and dehydroallogibberic acids, respectively.

LITERATURE CITED

1. J. F. Graebe and H. J. Roberts, Gibberellins, in: Phytohormones and Related Compounds: A Comprehensive Treatise, Biomed Press, Amsterdam, Vol. 1 (1978), p. 107.
2. I. Kuhr, Folia Mikrobiol, 7, No. 6, 358 (1962).
3. R. J. Pryce, Phytochemistry, 12, No. 3, 507 (1973).
4. R. J. Pryce, J. Chem. Soc. Perkin Trans. I, No. 10, 1179 (1974).
5. G. I. Baram, M. A. Grachev, N. A. Komarova, M. P. Perelroyzen, Ju. A. Bolvanov, S. V. Kuzmin, V. V. Kargaltsev, and E. A. Kuper, J. Chromatogr., 264, No. 1, 69 (1983).
6. H. A. Brune, U. Günter, P Lach, and R. Sihler, Z. Naturforsch., 40b, No. 6, 833 (1985).
7. H. K. Al-Ekabi and G. A. Derwish, Can. J. Chem., 62, No. 10, 1996 (1984).
8. R. B. Woodward, and R. Hoffmann, The Conservation of Orbital Symmetry, Academic Press, New York (1970) [Russian translation, Mir, Moscow (1971), p. 126].
9. F. G. Fiser, K. Löwenberg, Ber., 66, No. 5, 669 (1933).
10. M. Vilkas, N. A. Abraham, and J. Candehore, Bull. Soc. Chim. France, No. 6, 1196 (1960).
11. P. W. Brian, J. F. Grove, H. G. Hemming, T. P. C. Mulholland, and M. Radley, Plant Physiol., 33, No. 5, 329 (1958).
12. G. S. Muromtsev and V. N. Agnistikova, Gibberellins [in Russian], Nauka, Moscow (1984), p. 120.
13. B. E. Gross, J. F. Grove, and A. Morrison, J. Chem. Soc., No. 6, 2498 (1961).
14. J. F. Grove and T. P. C. Mulholland, J. Chem. Soc., No. 7, 3007 (1960).
15. A. J. Gordon, and R. A. Ford, The Chemist's Companion, Wiley-Interscience, New York (1972) [Russian translation, Mir, Moscow (1976), p. 447].