# A NEW PLANT GROWTH REGULATOR OF MICROBIAL ORIGIN

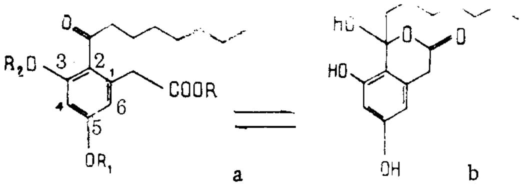
V. D. Voblikova, N. S. Kobrina, N. M. Gerasimova,
Z. N. Pavlova, G. F. Dem'yanova, V. P. Murygina,
L. I. Volosova, and G. S. Muromtsev

UDC 547.918+576.8

A new plant growth regulator – an inhibitor of the germination of mustard seeds – has been isolated from a culture filtrate of a phytopathogenic fungus of the genus Phoma. The methyl and trimethylsilyl derivatives of this compound have been obtained. Its empirical formula – $C_{16}H_{22}O_5$ – has been determined. On the basis of the results of IR and NMR spectroscopy and mass spectrometry, the compound isolated has been assigned the structure of 3,5-dihydroxy-2-octanoylphenylacetic acid.

It is known that microorganisms are capable of synthesizing various physiologically active substances. Particularly characteristic of phytopathogenic fungi is the capacity for synthesizing metabolites possessing growth-regulating activity [1]. A phytopathogenic fungus of the genus Phoma has been isolated from an infected section of an apple-tree branch. The physiological activity of its culture solution, of extracts, and of isolated metabolites on plants has been studied [2].

In the present paper we describe the isolation of a new inhibitor of the germination of plant seeds – 3,5-dihydroxy-2-octanoylphenylacetic acid (I), which is produced by this fungus. From a filtrate of the culture liquid of the fungus we isolated the crystalline substance (I) with a molecular weight of 294 and the elementary composition $C_{16}H_{22}O_5$.



I. $R_1 = R_2 = R = H$
II. $R = CH_3$, $R_1 = R_2 = H$
III. $R_1 = R_2 = R = Si(CH_3)_3$

3,5-Dihydroxy-2-octanoylphenylacetic acid and its derivatives.

The IR spectrum characterized the presence in the molecule of an aromatic ring (ν 1615 > 1515, 1970 $cm^{-1}$) and a hydroxy group (ν 3390 $cm^{-1}$). An absorption band at ν 1710 $cm^{-1}$ was characteristic for the vibrations of a C═O group, and a band at 2527 $cm^{-1}$ corresponded to an HO group. A diffuse band at 1655-1635 $cm^{-1}$ showed the presence of a carbonyl grouping. Substituents in an aromatic ring were characterized by CH vibrations at ν 800-830 $cm^{-1}$. The presence of phenolic hydroxyls in the substance was confirmed by a displacement of the absorption maximum in the UV spectrum on alkalinization from λ 302 nm in the long-wave direction (λ 326 nm).

In the mass spectrum of the metabolite (I), together with the peak of the molecular ion $M^+$ having m/z 294 and the peaks of the ions $(M - H_2O)^+$ with m/z 276 and $(M - CO)^+$ with m/z 250, there were intense peaks of ions with m/z 205, 195, 177, and 151. Metastable peaks confirmed the transformations of a number of ions 294 → 276 (m/z 295), 276 → 258 (m/z 241), and 195 → 167 (m/z 143). The elementary compositions of the fragmentary ions were determined by high-resolution mass spectrometry. The decomposition of the ions with m/z 294, 276, and 250 took place in accordance with the fragmentation of aryl alkyl ketones, with γ-hydrogen atoms in the side chains [3]. An ion with m/z 195 ($C_9H_7O_5$) was formed as the result of the

---

All-Union Scientific-Research Institute of Applied Molecular Biology and Genetics. V. I. Lenin Academy of Agricultural Sciences, Moscow. Translated from Khimiya Prirodnykh Soedinenii No. 3, pp. 387-391, May-June, 1985. Original article submitted May 30, 1984.

0009-3130/85/2103-0362$09.50

elimination of a $C_7H_{15}$ radical by the molecular ion. The elimination of CO by the on with m/z 195 showed that the ketone group was linked to a hydrocarbon radical.

The signals of the methylene protons of an aliphatic chain in the 2.87 ppm region of the PMR spectrum confirmed the presence of a carbonyl group attached to an aromatic nucleus. Because of hindrance to the rotation of the side chain, the protons of the $CH_2$ group in the α position to the carbonyl group gave a doubled doublet. The protons of the aromatic ring were represented by a two-proton singlet in the 6.3 ppm region. In the PMR spectrum of the methyl ester (II) the aromatic rings gave a broadened singlet with a coupling constant close to that of the meta interaction of protons in benzene, 0.5-1 Hz.

The methylene protons of the hydroxycarbonylmethyl group of (I) appeared in the form of a two-proton singlet at $\delta$ 3.68 ppm, and in the methyl ester (II) they appeared at 3.83 ppm. The presence of this grouping in the molecules of (I) was also confirmed by the presence of an ion with m/z 235 ($C_{14}H_{19}O_3$) in its mass spectrum.

When (I) was heated in a tube in the source of a mass spectrometer at 70°C for 5 min, it underwent complete decarboxylation and formed a substance with a molecular ion having m/z 250. The chemical-ionization spectrum of this substance contained only the peak of the $MH^+$ ion with m/z 251. In the spectrum of the acid (I) by chemical ionization with ammonia, however, in addition to the peaks of the quasimolecular ions $(MH)^+$ and $(M + NH_4)^+$ there were also the peaks of the ions $(MH - H_2O)^+$, $(MH - CO_2)^+$, and $(M + NH_4 - H_2O)^+$ and $(M + NH_4 - CO_2)^+$.

The silylation of compound (I) gave derivative (III) containing three $Si(CH_3)_3$ groups, which showed the presence of two hydroxy groups as well as the carboxylic OH group. The mass spectrum of (III) contained the peaks of the molecular ion $M^+$ with m/z 510 and of the ions $(M - CH_3)^+$ with m/z 495, $(M - CH_3 - C_6H_{12})^+$ or $(M - C_{17}H_{15})^+$ with m/z 411, $(M - CO - C_7H_{14})^+$ with m/z 384. The ion with m/z 411 eliminated a silanol molecule, which showed the presence of a $CH_2-COO-Si(CH_3)_3$ grouping in it, since the elimination of a $\dot{O}Si(CH_3)_3$ radical is characteristic for ethylmetnylsilylphthalate [4, 5].

The combination of these facts permitted us to consider that the compound was 3,5-dihydroxy-2-octanoylphenylacetic acid.

It may be assumed that acid (I) is present in two tautomeric forms – linear (a) and cyclic (b) (see formulas) – which is characteristic for compounds of a similar type [6]. The existence of two tautomeric forms of (I) is shown by the elimination of water from $M^+$ at the expense of the carboxy and ketone groups, as an investigation of the methyl ester (II) showed. The decomposition of acid (I) and its ester (II) on electron impact was similar to the decomposition of a compound with a similar structure – curvularin [7].

The 3,5-dihydroxy-2-octanoylphenylacetic acid isolated, in a concentration of 3 mg/liter, suppressed the germination of mustard seeds by approximately 50% (control, water – 100 %):

| | | | |
|---|---|---|---|
| Concentration of (I), mg/liter | 0.3 | 3.0 | 30.0 |
| Germination, % | 82.7 | 52.7 | 32.2 |

## EXPERIMENTAL

IR spectra were recorded on a Specord 75 IR spectrometer in paraffin oil, PMR spectra on a Varian XL-100A instrument with a frequency of 100 Hz in $(CD_3)_2CO$, and UV spectrum on a Spectord UV-Vis instrument in methanol. The TMS derivative was anlayzed on an LKB-9000 Chromass at an energy of the ionizing electrons of 70 eV. Mass spectra by electron impact and by chemical ionization with ammonia were obtained on an MAT-44S instrument at an ammonia pressure of 0.3 torr and a temperature of the ionization chamber of 190°C.

Isolation of 3,5-Dihydroxy-2-octanoylphenylacetic Acid (I). A filtrate of the culture liquid in an amount of 4.74 liters with pH 4.85 was acidified with 1 N $H_2SO_4$ to pH 3 and extracted with ethyl acetate (2 × 2 liters) with shaking on a shaking machine for 0.5 h. The ethyl acetate was evaporated off in vacuum on the water bath at 40°C to a volume of 500 ml. It was made alkaline to pH 8 with 5% $NaHCO_3$ (3 × 150 ml) and the alkaline aqueous layer was separated off, acidified to pH 3, and extracted with ethyl acetate. The ethyl acetate reextract was dried over $Na_2SO_4$ and evaporated in vacuum at a water-bath temperature not exceeding 40°C. This gave 295 mg of a residue which was separated chromatographically on a column of silica gel (100-200 μm) at a ratio of substance to adsorbent of 1:30. Pure or mixed solvents with increasing polarity were used as eluents: hexane; hexane–benzene (1:1); benzene; hexane–chloroform (8:2), (1:1), and (3:7); and chloroform. The fractions eluted by chloroform (49 mg) inhibited the germination of mustard and lettuce seeds. After its preparative separation on a 20 × 20 cm plate (ICN Pharmaceuticals, GFR) having a layer thickness of 0.25 mm in the chloroform–isopropanol (9:1) system 35 mg of (I) was obtained in the form of a crystalline substance with mp 130°C. On TLC, compound (I) absorbed in UV light, appearing in the form of a blue spot, and on spraying with $H_2SO_4$ and heating at 100°C for 5 min it gave a stable bright yellow coloration.

IR spectra (in paraffin oil, $cm^{-1}$): 3390, 3155, 3070, 1710, 1660, 1605, 1270, 1170, 860, 830.

UV spectrum, $\lambda_{max}$, nm (ε, MeOH): 221 (12,640), 231 (9850), 270 (6390), 302 (4780).

UV spectrum, $\lambda_{max}$, nm (C=O, 1 N NaOH): 253 (6760), 326 (17,350).

Electron-impact mass spectrum, (m/z density %), (elementary composition): 294 [$M^+$] (25) ($C_{16}H_{22}O_5$); 276 (43); 250 (23); 235 (15) ($C_{14}H_{19}O_3$); 233 (8) ($C_{11}H_{11}O_5$); 219 (12), 210 (18); 206 (43); 205 (80) ($C_{11}H_9O_4$); 195 (80) ($C_9H_7O_5$); 192 (50); 179 (27); 177 (70) ($C_9H_5O_4$); 168 (45); 167 (95) ($C_8H_7O_4$); 166 (52); 151 (100) ($C_8H_7O_3$); 150 (75); 138 (20); 137 (18); 123 (33); 121 (30).

Chemical ionization mass spectrum with ammonia, m/z (%): 312 (27) $[M + NH_4]^+$; 295 (100) $[M + H]^+$; 294 (47); 277 (18) $[M + H - H_2O]$; 251 (50) $[M + H - CO_2]^+$; 250 (21).

PMR spectrum, δ, ppm: 0.87 (3 H, t-m); 1.70 (2 H, t-t); 2.87 (d-d); 3.68 (2 H, s); 6.30 (2 H, s).

<u>Preparation of Methyl 3,5-Dihydroxy-2-octanoylphenylacetate (II).</u> An ethereal solution of diazomethane was added to a solution of 20 mg of (I) in 3 ml of ether until a permanant yellow coloration remained. The solution left to stand overnight. The substance was separated by preparative chromatogrpahy on a plate (20 × 20 cm) with a layer of silica gel in the chloroform–isopropanol (9:1) system. Four substances were isolated, with Rf 0.89, 0.57, 0.82, and 0.49. The substance with Rf 0.49 consisted of methyl 3,5-dihydroxy-2-octanoylphenylacetate (II).

Electron-impact mass spectrum, m/z (intensity %): 308 [$M^+$] (49); 290 (14); 277 (22); 253 (8); 249 (17); 237 (23); 235 (12); 224 (25); 223 (27); 219 (18); 209 (66); 206 (13); 205 (40); 195 (25); 192 (14); 182 (50); 181 (100); 177 (70); 165 (24); 164 (32); 163 (32); 151 (68); 150 (43); 149 (15); 137 (26); 135 (15); 126 (33); 123 (21); 121 (61); 109 (11); 107 (13); 95 (14); 81 (17); 73 (15); 71 (12); 69 (50).

PMR spectrum, $(CD_3)_2CO$, δ, ppm: 0.87 (3 H, t-m); 1.70 (2 H, d-d); 2.87 (2 H, d-d); 3.73 (3 H, s); 3.83 (2 H, s); 6.30 and 6.13 (J = 0.5-1 Hz).

<u>Preparation of the Trimethylsilyl Derivative (III).</u> N,N-Bis(trimethylsilyl)fluoroacetamide was added to (I). The product was analyzed on the Chromass using a 0.12 × 0.002 m glass column filled with Chromosorb W (100-200 mesh) impregnated with 2% of JXR at a rate of rise of temperature of 12°C per minute from 90 to 300°C. Compound (III) issued on the chromatogram in the form of a distinct single peak after 9 min.

Electron-impact mass spectrum, m/z (intensity, %): 510 (8): 496 (7); 495 (11); 492 (2), 485 (1); 477 (2); 476 (3); 438 (2); 426 (4); 420 (5); 412 (8); 411 (37); 410 (100); 405 (6); 392 (5); 384 (27); 349 (8); 322 (10); 321 (30); 295 (10); 294 (12); 265 (7); 219 (3); 147 (10); 133 (7); 109 (5); 73 (45); 69 (25); 51 (1).

The mass spectra of the trimethylsilyl derivative (III) was recorded with the direct introduction of the substance into the source, m/z (intensity, %): 510 (13); 496 (11); 495 (28); 485 (3); 439 (2); 426 (7); 420 (6); 413 (9); 412 (35); 411 (100); 405 (5); 395 (6); 385 (19); 384 (49); 349 (10); 322 (13); 321 (45); 295 (18); 294 (16); 265 (11); 147 (17); 133 (7); 109 (6); 73 (68).

<u>Decarboxylation of (I).</u> The acid (I) was heated at 70°C directly in the source of the mass spectrometer for 5 min. The spectrum of the substance formed by chemical ionization with ammonia contained only the peak of the $(M + H)^+$ ion with m/z 251.

The authors thank É. P. Serebryakov and B. V. Rosynov for taking the spectra and for assistence in interpreting the results.

## SUMMARY

A new plant growth regulator — 3,5-dihydroxy-2-octanoylphenylacetic acid, a seed germination inhibitor — has been isolated from the culture filtrate of a phytopathogenic fungus of the genus Phoma.

## LITERATURE CITED

1. G. S. Muromtsev, N. M. Gerasimova, N. S. Kobrina, V. M. Koreneva, and V. S. Smolyakov, Usp. Mikrobiol., 19, 106 (1984).
2. G. S. Muromtsev, N. M. Gerasimova, N. S. Kobrina, V. P. Murygina, Z. N. Pavlova, L. I. Volosova, V. D. Voblikova, and G. F. Dem'yanova, in: Plant Growth and Development Regulators. Abstracts of Lectures at the First All-Union Conference, Moscow, October 12-14, 1981 [in Russian], Moscow (1981), p. 203.
3. H. Budzikiewicz, C. Djerassi, and D. Williams, Interpretation of Mass Spectra of Organic Compounds, Holden-Day, San Francisco (1964).
4. E. Ende and G. Spiteller, Mass Spectrum Rev., 1, 29 (1982).
5. G. C. Smith and C. Djerassi, Org. Mass Spectrum, 5, 505 (1971).
6. K. Kameda, H. Aoki, H. Tanaka, and M. Nainaki, Agr. Biol. Chem., 37, No. 9, 2137 (1973).
7. H. D. Munro, O. C. Musgrave, and R. Templiton, J. Chem. Soc., 10 (1967).

---

# INFLUENCE OF ADDITIONS OF ANTHRAQUINONE ON THE ALKALINE HYDROLYSIS OF COTTON-PLANT LIGNIN

E. N. Yanishevskaya and Kh. A. Abduasimov

UDC 547.992:002.61

The action of catalytic amounts of anthraquinone on the alkaline hydrolysis of the stems and dioxane lignin of the cotton plant of the ssp. mexicanium has been investigated. It has been established that this leads to a substantial increase in the yield of low-molecular-weight hydrolysis products. Analysis of the monomeric fractions of the hydrolysis products by the GLC method has shown the presence in them of large amounts of substances with a p-coumaryl structure. This, and also the low level of syringyl derivatives, may be caused by the demethoxylation of the lignin under the conditions described.

The addition of catalytic amounts of anthraquinone (AQ) in the akaline digestion of wood leads to an appreciable increase in the rate of delignification through an increase in the breakdown of the β-ethers of units with free phenolic hydroxy groups [1, 2]. The mechanism of the cleavage with the participation of AQ of β-OH ether bonds between guaiacyl structural units forming the lignin of conifer wood has been studied in detail with the aid of experiments on model compounds [3]. The action of AQ on the lignin of the wood of broad-leaved trees, which also includes syringyl units, has not been studied.

We have investigated the influence of small additions of AQ on the alkaline hydrolysis of the stems and the dioxane lignin (DLA) of the cotton plant ssp. mexicanium. The DLA contained about 0.5% of carbohydrates the presence of which is necessary for the inclusion of AQ in the redox cycle [4] and which makes it effective in catalytic amounts.

Hydrolysis was performed in 8% NaOH solution in a nitrogen atmosphere at various temperatures with various amounts of AQ, and also without it. To facilitate the identification of the low-molecular-weight products formed, the reaction mixture was reduced with Raney nickel. After acidification to pH 7-8 the monomeric fraction of the hydrolysis products was extracted with ether, and the residual low-temperature-weight substances were extracted with ethyl acetate at pH 2.

---

Institute of the Chemistry of Plant Substances, Academy of Sciences of the USSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 391-395, May-June, 1985. Original article submitted July 3, 1984.

0009-3130/85/2103-0365$09.50