USE OF HIGH-SPEED LIQUID CHROMATOGRAPHY FOR SEPARATING THE GIBBERELLINS AND COMPOUNDS RELATED TO THEM

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Known methods of obtaining 4-bromophenacyl esters of the gibberellins are considered. A convenient method of obtaining 4-bromophenacyl esters of gibberellins, which are readily recorded by the UV detector of a liquid chromatograph, is described. The chromatographic separation has been performed of two model mixtures of esters of different polarities on fine-grained silica gel.

The gibberellins, natural phytohormones possessing a complex structure, are compounds that have close physicochemical properties. Consequently, apart from the study of their chemical transformations and biological functions, intensive investigations are being performed on their separation and analysis. Up to the present time, methods for the separation of the gibberellins with the aid of gas–liquid chromatography (GLC) [1], thin-layer chromatography (TLC) [2], and column chromatography [3] have been well developed. Of these methods only the first is applicable to the quantitative analysis of the small samples that are usually available to one investigating the endogenous gibberellins of plants. However, the GLC method has a disadvantage which consists in the possibility of the thermal decomposition of the components of the sample during chromatography. The method of high-speed liquid chromatography (HSLC) is free from this disadvantage, since separation by its use takes place at room temperature. The method of HSLC has been used successfully for the fractionation of cytokinins [4], prostaglandins [5], and many other compounds. The use of this method for the analysis of gibberellins has been described only by Morris [6], who used the 4-bromophenacyl esters of the gibberellins for analysis. These esters are very convenient for analysis by the HSLC method, since in liquid chromatographs UV detectors recording the absorption in the 254 nm region are usually used. Strong absorption at 256 nm is characteristic for 4-bromophenacyl esters. The presence of such an absorbing group in various gibberellins can considerably simplify the treatment of the chromatograms.

At the present time there is no simple and convenient method for obtaining 4-bromophenacyl esters of the gibberellins. Thus, Cross [7] used Umeh's method [8] and obtained the 4-bromophenacyl ester of gibberellin A_3, but under the reaction conditions the isomerization of gibberellin A_3 into gibberellin iso-A_3 is possible and was in fact detected by Cross in one of his experiments. According to Durst's method [9], which was used by Morris [6], the reaction is carried out with the sodium salts of the gibberellins, which in themselves are difficultly accessible because of the tendency of some gibberellins to undergo isomerization.

We have found that the 4-bromophenacyl esters of the gibberellins can be obtained in quantitative yield by a method similar to that used by Moreland [10] for the synthesis of the 4-bromophenacyl esters of acetic and benzoic acids. The reaction of a gibberellin with 4-bromophenacyl bromide in acetone solution at a temperature of about 45°C in the presence of triethylamine is usually complete after one hour. By this method we have synthesized the 4-bromophenacyl esters of gibberellin A_3 (I), 3-O-acetylgibberellin A_3 (II), 3,13,-di-O-acetylgibberellin A_3 (III), gibberellin A_9 (IV), and the dicarboxylic acid (V) obtained by the hydrolysis of fujenal (VI). Fujenal and the corresponding dicarboxylic acid accompany the gibberellins in mixtures of metabolites of the fungus *Fusarium moniliforme* [11] – the industrial producing agent of the gibberellins.

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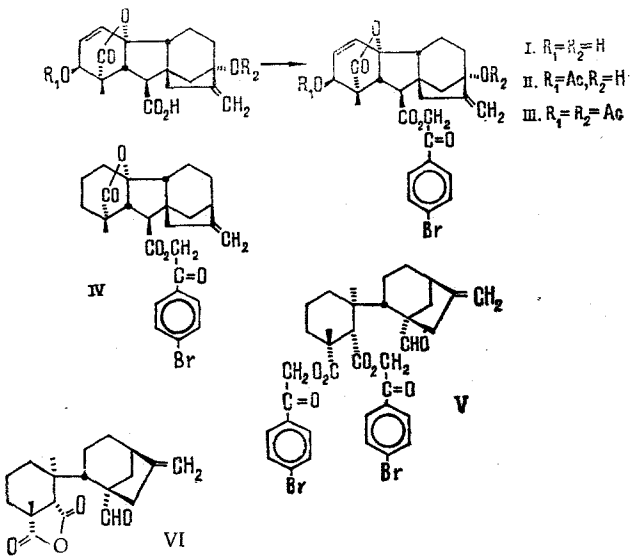

To separate the 4-bromophenacyl esters of gibberellins, Morris [6] used chemically modified cyanopropyl and octadecyl silica gels which are phases of medium and low polarity, respectively, as the stationary phases. Such chemically bound phases are not readily available.

We have found that it is possible to use a polar phase — fine-grained silica gel — for the separation of the 4-bromophenacyl esters of the gibberellins. The most convenient mobile phase that has little absorption in the 254 nm region and is stable on storage proved to be a mixture of methylene chloride and acetonitrile. For the chromatographic separation of the esters (I), (II), and (III) on fine-grained silica gel the optimum volume ratio of methylene chloride and acetonitrile is 10:1. At a rate of flow of 0.5 ml/min in a column 50 cm long with a diameter of 2.6 mm the retention times for these compounds are, respectively, 21.0, 11.1, and 7.5 min. A mixture of compounds (IV) and (V) was analyzed with the addition of 70-80% of hexane to the mobile phase. At a rate of flow of 0.5 ml/min (70%) of hexane) no separation was observed, the retention time being 13.4 min. Complete separation was achieved only with a decrease in the rate of flow to 0.2 ml/min and the adjustment of the proportion of hexane in the mobile phase to 77%. The retention times of the model compounds analyzed under these conditions were 37.9 and 31.0 min, respectively.

The minimum amount of compound with one 4-bromophenacyl group that can be determined is about 5 nanograms, and with two such groups about 2.5 nanograms.

EXPERIMENTAL

Chromatography was carried out in a Perkin-Elmer 1220 liquid chromatograph with a UV detector (253.7 nm) and a column 50 cm × 2.6 mm filled with Sil-X-I fine-grained silica gel. The solvents used in the investigation were previously distilled and filtered in vacuum through a porous glass filter.

The IR spectra were taken of solutions in chloroform on a UR-20 instrument and the molecular weights and elementary compositions of the compounds synthesized were determined by high-resolution mass spectrometry on a MS-902 instrument. The purity of the substances and the course of the reactions for preparing the 4-bromophenacyl esters were examined by the TLC method on Silufol plates in the chloroform-methanol (20:1) system.

3β,10,13-Trihydroxy-20-norgibberella-1,16-diene-7,19-dioic Acid 7-(4-Bromophenacyl) Ester 19,10-Lactone (I). A suspension of 2.92 g of gibberellin A_3 in 30 ml of acetone was treated first with 1.2 ml of triethylamine and then with 2.50 g of 4-bromophenacyl bromide. The solution was kept at 45°C for 1 h and was then poured with stirring into 200 ml of water. After 1 h the precipitate was filtered off and was dried over phosphorus pentoxide, giving 4.45 g of compound (I). The spectral characteristics and melting points corresponded to those described in the literature [7].

3β-Acetoxy-10,13-dihydroxy-20-norgibberella-1,16-diene-7,19-ioic Acid 7-(4-Bromophenacyl) Ester 19, 10-Lactone (II). A suspension of 4.21 g of gibberellin A_3 acetate in 20 ml of acetone was treated with 1.68 ml of triethylamine and a solution 3.17 g of 4-bromophenacyl bromide in 20 ml of acetone. The reaction mixture was left overnight, and then the solution was evaporated to dryness. The residue was dissolved in ethyl acetate, the salt was filtered off, and the ethyl acetate solution was evaporated to give 6.09 g of compound (II) with mp 183-185°C. IR spectrum $\nu_{max}^{CH_3Cl_3}$, cm^{-1}: 1780 (lactone), 1745 (O–C=O), 1750 (C=O), 1670 (C=C). UV spectrum, $\lambda_{max}^{C_2H_5OH}$, nm: 256 (log ε 4.32). The analysis of compound (II) corresponded to calculated figures. Found: mol. wt. 584 (mass spectrometry). $C_{29}H_{29}O_8Br$. Calculated: mol. wt. 585.4.

3β-13-Diacetoxy-10-hydroxy-20-norgibberella-1,16-diene-7,19-dioic acid 7-(4-bromophenacyl) ester 19,10-lactone (III) was obtained similarly with mp 164-166°C, IR spectrum, $\nu_{max}^{CHCl_3}$, ester 19,10-lactone (III) was obtained similarly with mp 164-166°C, IR spectrum, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1780 (lactone), 1745 (O–C=O), 1705 (C=O), 1670 (C=C). UV spectrum $\lambda_{max}^{C_2H_5OH}$, nm: 256 (log ε 4.29). Found: mol. wt. 626.1151 (mass spectrometry). $C_{31}H_{31}O_9Br$. Calculated: mol. wt. 626.1158.

10-Hydroxy-20-norgibberella-16-ene-7,19-dioic acid 7-(4-bromophenacyl) ester 19-10-lactone (IV) was obtained similarly. IR spectrum, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1780 (lactone), 1750 (O–C=O), 1705 (C=O), 1670 (C=C). UV spectrum, $\lambda_{max}^{C_2H_5OH}$, nm: 256 (log ε 4.29). Found: mol. wt. 512.1198 (mass spectrometry). $C_{27}H_{29}O_5Br$. Calculated: mol. wt. 512.1185.

1α,2α-Di(4-bromophenacyloxycarbonyl)-3β-(1α-formyl-6-methylenebicyclo[3.2.1]oct-2α-yl)-1β,3α-dimethylcyclohexane (V), was obtained similarly, with mp 145-147.5°C. IR spectrum, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1745 (O–C=O), 1705 (C=O), 1680 (C=C), UV spectrum, $\lambda_{max}^{C_2H_5}$, nm: 256 (log ε 4.54). Found: mol. wt. 740.0969 (mass spectrometry). $C_{36}H_{38}O_7Br_2$. Calculated: mol. wt. 740.0983.

The dicarboxylic acid for the synthesis of compound (V) was obtained by the hydrolysis of 1.69 g of fujenal (VI) in a mixture of 40 ml of water and 10 ml of tetrahydrofuran in the presence of 5 g of potassium hydroxide for 2 h. The solution was acidified to pH 2, and the dicarboxylic acid was extracted with ethyl acetate.

SUMMARY

4-Bromophenacyl esters of the gibberellins are obtained smoothly in acetone by treatment of the acids with triethylamine and 4-bromophenacyl bromide.

To analyze the mixtures of acidic metabolites of the fungus *Fusarium moniliforme* in the form of 4-bromophenacyl ethers by high-speed liquid chromatography it is possible to use fine-grained silica gel as the stationary phase.

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