BITTER SUBSTANCES OF CUCUMBERS

I. PARTIAL STRUCTURE OF CUCURBITACIDE E

P. K. Kintya, N. E. Isaeva,
V. Ya. Chirva, and G. V. Lazur'evskii

UDC 547.913+547.918

The bitter substances of the gourd family, Cucurbitaceae, have been known for a long time, but their chemical nature has been investigated only in recent years. It has been established that the bitterness is due to the presence in the plants of this family of peculiar tetracyclic triterpenoids – cucurbitacins – which are present in them both in the free state and in the form of glycosides. Several dozens of cucurbitacins differing in the number, nature, and arrangment of the functional groups in the carbon skeleton have been described [1].

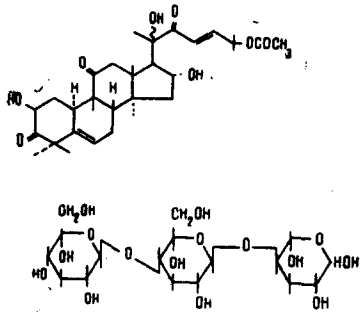
From the leaves and fruit of cucumbers (Cucumis sativus) free cucurbitacins B and C have been isolated. But there is no information in the literature on the amount of these cucurbitacins in cucumbers. In cucumber seeds we have found five glycosides differing in chromatographic mobility. The most polar of them, which has been called cucurbitacide E has been isolated in the pure state, and a partial structure has been determined for it.

By paper chromatography, an acid hydrolyzate of the glycoside was found to contain glucose and arabinose, present, according to the results of photocolorimetry, in a ratio of 2:1. The aglycone underwent degradation under these conditions. It was obtained in the native state by treating cucurbitacide E with an enzyme preparation from Rhizopus arrizus. The substance proved to be identical with cucurbitacin B [2].

An investigation of the products of the methanolysis of methylated cucurbitacide E by Hakomori's method [3] showed the presence of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, and 2,3-di-O-methyl-L-arabinose. The results of periodate cleavage confirmed the structure of these products.

The sequence of the monosaccharides was determined by the partial hydrolysis and enzymatic decomposition of the glycoside. In the first case, arabinosyl- and glucosylarabinosylcucurbitacin B were isolated and in the second case cellobiose.

Thus, cucurbitacide E has a structure in which one of the hydroxyls of the aglycone is substituted by a trisaccharide residue.



Institute of Chemistry, Academy of Sciences of the Moldavian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 306-309, May-June, 1972. Original article submitted December 24, 1971.

EXPERIMENTAL

Chromatography was performed on type "C" (fast) paper of the Volodarskii Leningrad mill, on silica gel of type KSK, and on alumina (Brockmann activity grade II).

The glycoside and the aglycone were revealed with a 2% ethanolic solution of vanillin in 85% orthophosphoric acid (1 : 2) [4], and also by means of conc. sulfuric acid. The sugars on the paper chromatograms were revealed with aniline phthalate and photocolorimetered on an FÉKN-57 instrument. Gas-liquid chromatography was performed on an LKhM-8M chromatograph with hydrogen as the carrier gas at a temperature of 160°C with 20% of Reoplex 400 on Chromosorb W, 60-80 mesh.

Isolation of Cucurbitacide E. Comminuted cucumber seeds (1.5 kg) were exhaustively extracted with 70% methanol in a Soxhlet apparatus. The extracts were evaporated to dryness in a rotary evaporator at 50°C. Then the product was dissolved in water, and the solution was washed successively with petroleum ether, diethyl ether, and chloroform. The aqueous layer was shaken repeatedly with butanol, after which the organic extracts were evaporated to dryness. Yield 1.7 g.

The extract (500 mg) was deposited on a column (60 × 4 cm) and was eluted in the chloroform–methanol–water (61 : 32 : 7) system. The fractions containing pure cucurbitacide E (I) were combined and evaporated. Yield 0.25 g, mp 158-160°C.

Identification of the Aglycone and the Monosaccharides. A. A solution of 50 mg of cucurbitacide E in 5 ml of 1% H_2SO_4 was heated in a sealed tube at 100°C for 3 h. Then the contents of the tube were diluted with water and extracted with ether. The hydrolyzate remaining after the separation of the ethereal layer was deionized with KU (H^+ form) and AV-17 (OH^- form) ion-exchange resins. The resins were filtered off and washed with water, and the solution was evaporated and investigated for sugars by paper chromatography in the butanol–benzene–pyridine–water (5 : 1 : 3 : 3) system. Arabinose and glucose were found. The amounts of the monosaccharides were determined by comparing the results of the photocolorimetry of eluates of the spots formed by the monosaccharides when the paper chromatograms were treated with aniline phthalate. The glucose and arabinose were present in a ratio of 2.1 : 1.

B. A solution of 50 mg of the cucurbitacide in water was treated with an enzyme preparation from Rhizopus arrizus, heated at 35°C for 3 h, and extracted with ether. A substance was obtained which, after recrystallization from methanol, had mp 176-178°C and $[\alpha]_D$ + 84.3° (c 1.5; ether). The compound was identical in chromatographic behavior and IR spectra with cucurbitacin B, a sample of which was kindly given to us by Ya. V. Zielinsky (Gdansk, Poland). Literature data: mp 178-179°C, $[\alpha]_D$ + 87° [2].

Partial Hydrolysis of Cucurbitacide E. A mixture of 80 mg of the substance and 10 ml of 2.5% oxalic acid was heated at 78°C. After 3 h, two glycosides of different polarities were found in a sample by chromatography in a thin layer of silica gel in the chloroform–methanol–water (61 : 32 : 7) system. The individual substances were obtained by separation on a column of silica gel in the same system. On acid hydrolysis, from one of them arabinose was identified, and from the other arabinose and glucose.

Methylation of the Saponin. A mixture of 20 ml of dimethyl sulfoxide and 20 mg of sodium hydride was stirred in a current of argon until a dark green coloration had appeared (45 min). A solution of 50 mg of pure cucurbitacide E in the minimum volume of dimethyl sulfoxide was added to the methyl sulfoxide carbanion so obtained, and stirring was continued for 40 min. Then 10 ml of methyl iodide was added, and the mixture was left for 1 h.

The reaction product was diluted with water and exhaustively extracted with chloroform. The completeness of methylation was checked on alumina in the toluene–ethanol (9 : 1) system and by IR spectroscopy.

Hydrolysis of Methylated Cucurbitacide E and Identification of the Methyl Glycosides. A mixture of 40 mg of the fully methylated cucurbitacide E and 40 ml of a mixture of absolute methanol and 72% perchloric acid (10 : 1) was heated in the water bath for 3 h. Then the solution was diluted with water, the resulting precipitate of the aglycone was filtered off, and the aqueous solution was neutralized with Dowex (HCO_3^- form) and IR-120 (H^+ form) resins.

The methylated methyl glycosides in the filtrate were identified by gas-liquid chromatography and by thin-layer chromatography in the benzene–acetone (2 : 1) system in the presence of authentic samples. 2,3,4,6-Tetra-O-methyl-D-glucose, 2,3,6-tri-O-methylglucose, and 2,3-di-O-methylarabinose were identified.

Periodate Oxidation of the Saponin. Cucurbitacide E (15 mg) was added to 30 mg of $NaIO_4$ in 25 ml of water, and the mixture was left in the dark for three days. Then 0.1 ml of ethylene glycol was added, and after 1 h the solution was deionized. After concentration, there were no monosaccharides in the residue (chromatography).

Enzymatic Hydrolysis. To 250 mg of cucurbitacide E in 20 ml of phosphate buffer (pH 5.7) was added a few milligrams of diastase, and the mixture was kept at 30°C for 6 h. After the end of enzymatic hydrolysis, in addition to monosaccharides an oligosaccharide was found which was identical in chromatographic behavior on paper in the butanol–benzene–pyridine–water (5:1:3:3) system with cellobiose.

The oligosaccharide was isolated in the individual state by preparative paper chromatography and subjected to acid hydrolysis, after which only glucose was identified.

SUMMARY

Five tetracyclic triterpene glycosides – cucurbitacides – have been found in cucumber seeds.

The partial structure of cucurbitacide E has been established: it is a trioside of cucurbitacin B.

LITERATURE CITED

1. K. Blait, Postepy Nauk Roln., 3, 51 (1968).
2. W. Schlegel and C. R. Noller, Tetrahedron Lett., 1960, 15.
3. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
4. J. Zielinski and J. Konopa, J. Chromatogr., 36, 540 (1968).