FLAVONOIDS OF Bidens tripartita

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Continuing a study of the flavonoid composition of the herb Bidens tripartita L. (bur beggarticks), family Compositae, and making use of chromatography on polyamides, we have obtained, in addition to the substance A isolated previously [1], two more flavonoids, B and C (Table 1).

Flavonoid B, $C_{15}H_{12}O_5$, appears on a chromatogram in UV light in the form of a dark brown spot becoming red on treatment with ammonia vapor or with 10% aqueous methanolic alkali. Qualitative reactions (see Table 1), and also the considerably greater intensity of the maxima of band I in a neutral solution plus zirconyl salts, alkali, sodium acetate, and boric acid (3',4'-OH) in contrast to the maximum of band II of a neutral solution plus sodium acetate (4-OH) shows that the compound isolated is a chalcone [2, 3]. The chalcone nature is also confirmed by the ready oxidation of substance B in an alkaline medium with atmospheric oxygen to an aurone and by its reduction in an acid medium on heating to a flavanone.

The alkaline degradation [4] of the compound under investigation formed resorcinol and protocatechiuc acid. This shows that the hydroxy groups are present in the 2,3',4,4' positions of the chalcone nucleus. The facts presented enable substance B to be characterized as 2,3',4,4'-tetrahydroxychalcone, or butein [5].

Flavonoid C, $C_{15}H_{10}O_5$, appeared on a chromatogram in UV light as a bright yellow-green spot acquiring a crimson color on treatment with ammonia vapor or alkali. If higher concentration of alkali (50%) was used, the color changed to lilac.

The IR spectra of the substance with additives (Table 2) showed that the hydroxy groups were present in the 3',4',6 positions. This was confirmed by alkaline degradation, which gave resorcinol and protocatechuic acid.

The structure of this aurone was shown by comparison with the product of the oxidation of the chalcone C, which enabled it to be identified as 3',4',6-trihydroxyaurone or sulphuretin [5].

Substance A, $C_{21}H_{22}O_{10}$, did not fluoresce on a chromatogram in UV light, but after treatment with ammonia vapor or alkali a blood-red spot appeared. Analysis of its UV spectra (Table 2) and a positive borohydride reaction [6] showed that the substance belonged to the class of flavanones. In a neutral solu-

TABLE 1. Properties of the Flavonoids of Bidens tripartita

Substance	mp, °C	$[\alpha]_D^{18}$, deg	R_f	Color of a solution on reduction with		
				Mg+HCl	$NaBH_4$+HCl	KOH
Butin 7-O-D-glucopyranoside (A)	165–167	−78	0.75	Blue-violet	Greenish-blue	Blood red
2,3',4,4'-Tetrahydroxychalcone (B)	213–215	—	0.10	Negative		Red
3',4',6-Trihydroxyaurone	280–285	—	0.18			Crimson

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TABLE 2. Spectral Characteristics of the Flavonoids of Bidens tripartita

Substance	Neutral solution	CH_3COONa	$CH_3COONa + H_3BO_3$	$Zr(OCl)_2$	KOH
Butin 7-O-β-D-glucopyranoside	270 310	310	295	300	455
2,3',4,4'-Tetrahydroxychalcone	260 380	420	420	475	445
3',4',6-Trihydroxyaurone	255 380	425	440	435	455
Sulphuretin 6-O-β-D-glucopyranoside	275 400	400	450	445	490

tion of the glycoside, the intensity of the maximum of the short-wave band was considerably greater than that of the long-wave band. The addition of alkali led to an intense maximum at 445 nm, which is characteristic for chalcone derivatives having a 3',4'-dihydroxy grouping in ring B [7].

Flavanones with a free hydroxy group in the 4' position and an alkylated or glycosylated hydroxy group in position 7 are capable of very readily undergoing cleavage to chalcones, in consequence of which a large bathochromic shift of the maximum of band I takes place [8].

Concentrated solutions of alkalis cleave all flavanones to chalcones, but cleavage with dilute alkalis is characteristic only for the above-mentioned type of substitution [9].

Hydrolysis of the substance with rhamnodiastase gave D-glucose and an aglycone identical with the 3',4',7-trihydroxyflavanone (butin) reduced from substance B.

When substance A was oxidized to an aurone glycoside and was then hydrolyzed, an aglycone identical with sulphuretin was formed.

It was established by the spectrophotometric method [10] that substance A is a monoside.

The position of attachment of the glucose to the aglycone was determined by comparing the UV spectra of the aurone glycoside obtained by the oxidation of substance A, and sulphuretin. The absence of a bathochromic shift on the addition of sodium acetate in the case of the glycoside but not of the aglycone showed that the glucose was attached in position 6 of sulphuretin. Consequently, the glucose was attached to the butin in position 7.

The presence in the IR spectrum of the glycoside of three strong absorption bands in the 1010-1100 cm^{-1} region shows the pyranose form of the glucose [11], which was also confirmed by the rate of acid hydrolysis [12].

The splitting off of the glucose on hydrolysis with rhamnodiastase shows that it is attached to the aglycone by a β-glycosidic bond [13]. This is also confirmed by the negative value of the specific rotation and by IR spectroscopy [11].

Thus, substance A may be ascribed in the structure of butin 7-O-β-D-glucopyranoside.

EXPERIMENTAL

The following solvent systems were used for the analysis of the substances isolated: 1) 50% formic acid; 2) 2% acetic acid; 3) phenol saturated with water, on Filtrak No. 12 chromatographic paper. The UV spectra were recorded on an SF-4 spectrophotometer and the IR spectra on a UR-20 instrument. The optical rotations were measured on an SPU-M instrument. Melting points were determined on a Kofler block. The elementary analyses of all the compounds corresponded to the calculated figure.

Isolation of the Substances. The comminuted herb (2 kg) was treated three times with 50% ethanol and the resulting extract was evaporated in vacuum to a volume of 3.5 liters. The resinous deposit that formed was separated off. The extract was then evaporated to 1.6 liter and was left for 16 h. The amorphous precipitate was separated by centrifuging, and the filtrate was gradually mixed with acetone (tenfold amount). The resinous products that separated out, which did not contain flavonoids, were removed, and the solution was evaporated to a volume of 0.5 liter and mixed with 250 g of Kapron powder and the mixture was dried in the air and deposited on a column (d 8, h 55 cm).

Elution was performed first with chloroform and then with mixtures of chloroform and ethanol having increasing concentrations of ethanol. The separation of the substances and the subsequent selection of the fractions was monitored by fluorescence in UV light and by paper chromatography.

Alkaline Degradation of Substance B. A mixture of 5 mg of the compound and 0.2 g of caustic potash was fused for 1-2 min. Then it was dissolved in 10 ml of water, and the solution was neutralized with hydrochloric acid and extracted with diethyl ether (5 × 10 ml). After the evaporation of the ether, the dry residue was chromatographed on paper in system 2. The degradation of substances A and C was performed similarly.

Oxidation of Butein to Sulphuretin. A solution of 50 mg of the substance in 10 ml of methanol was treated with 0.5 ml of a saturated methanolic solution of ammonia and was left at room temperature for 30 min. Then the solution was evaporated in vacuum to dryness and the residue was crystallized from water. Substance A was oxidized similarly to sulphuretin 6-O-β-D-glucopyranoside (sulphurein).

Reduction of Butein to Butin. A solution of 50 mg of the flavonoid in 20 ml of 50% methanol was treated with sufficient hydrochloric acid to give a 5% concentration of it in the solution after the methanol had been evaporated off. Then the reaction mixture was heated at 100°C for 2 h. After 45 min, it deposited colorless acicular crystals of butin.

Enzymatic Hydrolysis of the Butin Glycoside. A solution of 5 mg of the compound in 1 ml of water was treated with 5 mg of rhamnodiastase suspended in 1 ml of water, and the reaction mixture was left in a thermostat at 38°C for 12 h.

The presence of butin was shown by paper chromatography in systems 1 and 2 and that of D-glucose in system 3.

Acid Hydrolysis of the Butin Glycoside. A solution of 10 mg of the substance in 5 ml of 2% aqueous hydrochloric acid was heated in the boiling water bath for 3 h.

The butin glycoside had undergone complete hydrolysis in 2 h.

The aglycone was extracted with ethyl acetate, the extracts were combined and evaporated to dryness, and the residue was identified by chromatography on paper in system 1 as butin.

The aqueous extract was neutralized with AV-17 anion-exchange resin (OH form), evaporated to 0.5 ml, and chromatographed in system 3.

D-Glucose was obtained.

Sulfurein was hydrolyzed similarly. Sulphuretin and D-glucose were found in the hydrolyzate.

SUMMARY

The herb Bidens tripartita L. has yielded a chalcone identified as butein, an aurone identified as sulphuretin, and a new flavonoid butin 7-O-β-D-glucopyranoside.

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