by chromatography on silica gel with hexane—acetone (7:3) were isolated crystals with the composition $C_{15}H_{24}O_4$, mp 228-230°C (ethanol), 20 mg, $M^+$ 268.

## SUMMARY

1. A new lactone, isoridentin, with the composition $C_{15}H_{20}O_4$, has been isolated from the epigeal part of *Achillea biebersteinii*.

2. On the basis of chemical and spectral information it has been established that isoridentin has the structure of 1,3-dihydroxygermacra-4,10(14),11(13)-trien-6,12-olide.

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# UV-SPECTROPHOTOMETRIC DETERMINATION OF GLYCYRRHIZIC ACID IN *Glycyrrhiza glabra*

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The analysis of glycyrrhizic acid in licorice has been discussed in the literature [1-5]. The methods proposed by the authors of these papers are based on the gravimetric, titrimetric, or photocolorimetric determination of glycyrrhetic acid (genin), isolated in the hydrolysis of glycyrrhizic acid [1, 4-8]. These methods are associated with considerable expenditure of time, laboriousness, and nonspecificity, since accompanying substances are analyzed in addition to the glycyrrhizic acid. The authors of these methods are faced with the necessity of choosing the optimum conditions and for checking the completeness of hydrolysis.

Gravimetric methods in which the determination is effected from the weight of the crude glycyrrhizic acid have also found practical use (State Pharmacopoeia of the USSR, Tenth ed., 1968; Pharmacopoeia of the German Democratic Republic, Seventh ed., 1970) [1-3].

Recently, chromatographic methods of determining glycyrrhizic acid with the aid of chromatography in a thin layer of sorbent (TLC) have been developed which permit this acid to be analyzed without the accompanying components [9, 10].

We have developed a method for analyzing glycyrrhizic acid in roots and in the thick extract and dry licorice powder produced industrially. The proposed method is based on the chromatographic separation of glycyrrhizic acid from the accompanying substances followed by its spectrophotometric determination. For comparison, a thick licorice extract and a dry licorice powder were analyzed gravimetrically and by the methods developed (Tables 1 and 2).

As can be seen from Tables 1-2, the results obtained by the gravimetric method are high. A qualitative chromatogram in the system of solvents given below of the crude glycyrrhizic acid obtained by the gravimetric method showed the presence of four unidentified substances in addition to glycyrrhizic and glycyrrhetic acids. The $R_f$ value of glycyrrhizic acid is 0.33 and those of the closest unknown substances 0.23 and 0.45.

The chromatographic separation of the glycyrrhizic acid from the accompanying substances was effected in a fixed layer of silica gel in the chloroform—methanol—water (80:35:7) solvent system with a 1% solution of vanillin in sulfuric acid as the chromogenic agent; the sensitivity of the chromatographic method is 5 μg.

The UV spectrum of ammonium glycyrrhizate has one intense band with $\lambda_{max}$ 252 nm (log ε 4.04, $E^{1\%}_{1cm}$ = 130 ± 3.84). The intensity of this extremum enables it to be used as the analytical band. The absorption of solutions of the acid in the region of working concentrations (D = 0.1-0.6) obeys Beer's law.

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TABLE 1.

| Serial No. | Amount of glycyrrhizic acid (%) determined by the gravimetric method | Amount of glycyrrhizic acid (%) determined by the spectrophotometric method | Reproducibility of the spectrophotometric method |
|---|---|---|---|
| 1 | 21,6 | 15,7 | $\Sigma (x_{av}-x)^2=4,875$ |
| 2 | 20,4 | 16,9 | $Sn = \sqrt{\frac{\Sigma (x_{av}-x)^2}{n-1}} =$ |
| 3 | 21,4 | 14,8 | |
| 4 | 20,9 | 15,9 | $= \sqrt{\frac{4,875}{9}} = \pm 0,74$ |
| 5 | 21,8 | 16,4 | |
| 6 | 20,0 | 14,8 | $E_{rel} = \pm \frac{Sn \cdot t_\alpha n}{\sqrt{n}} =$ |
| 7 | 21,9 | 14,8 | |
| 8 | 21,3 | 15,6 | $= \pm \frac{0,74 \cdot 2,28}{\sqrt{10}} = \pm 0,534$ |
| 9 | 20,9 | 15,0 | |
| 10 | 19,8 | 15,5 | |
| | | $x_{av}=15,49$ | |

TABLE 2.

| Sample | Amount of glycyrrhizic acid (%) determined by the gravimetric method: found | average of two determinations | maximum deviation from the mean | Amount of glycyrrhizic acid (%) determined by the spectrophotometric method: found | average of two determinations | maximum deviation from the mean |
|---|---|---|---|---|---|---|
| Dry licorice powder (Soviet commercial brand) | 22,4<br>20,8 | 21,6 | ±3,70 | 13,00<br>12,85 | 12,925 | ±0,580 |
| C-FSD (American commercial brand) | 24,1<br>20,6 | 22,35 | ±7,80 | 10,50<br>9,90 | 10,200 | ±0,292 |
| A-USu (American commercial brand) | 23,2<br>20,5 | 21,85 | ±6,2 | 11,70<br>11,05 | 11,375 | ±0,290 |
| Dry licorice powder (sample from the Chinese Peoples' Republic) | 34,6<br>31,4 | 33,0 | ±4,85 | 13,40<br>14,00 | 13,700 | ±0,219 |

The glycyrrhizic acid is eluted from the silica gel by 50% (by volume) ethanol. The completeness of desorption of glycyrrhizic acid from silica gel has been confirmed by the chromatography of known amounts of a standard sample of ammonium glycyrrhizate followed by the elution of the spots and the spectrophotometry of the eluate at 252 nm. Desorption takes place satisfactorily and guarantees an error of within ±2.7% at 95% probability. The sensitivity of the chromatospectrophotometric method is 8 μg/ml.

The identity of an eluate of glycrrhizic acid on chromatographing a crude extract, dry powders, and licorice root with a standard sample of it was shown by IR and UV spectroscopy.

Licorice roots have also been analyzed by this method (Table 3).

The proposed method has good reproducibility for a thick extract (see Table 1): $E_{rel}$ = ±0.534. The maximum deviation from the mean for dry powders (see Table 2) did not exceed ±2.05%.

The accuracy of the method was confirmed by adding to the solution of thick extract under consideration (with a calculated glycyrrhizic acid content of 0.188 mg) a solution of a standard sample of ammonium glycyrrhizate:

| Ammonium glycyrhizate added, mg | Amount of glycyrrhizic acid with the addition, mg | Amount of glycyrrhizic acid with the addition found, mg | Glycyrrhizic acid added: mg | Glycyrrhizic acid added: % | Error of the analysis |
|---|---|---|---|---|---|
| 0,1098 | 0,298 | 0,299 | 0,111 | 101,1 | +1,1 |
| 0,1730 | 0,361 | 0,362 | 0,174 | 100,6 | +0,6 |
| 0,1730 | 0,361 | 0,370 | 0,182 | 105,2 | +5,2 |
| 0,1620 | 0,350 | 0,340 | 0,152 | 93,6 | −6,4 |
| 0,1290 | 0,317 | 0,317 | 0,125 | 96,9 | −3,1 |
| 0,1290 | 0,317 | 0,321 | 0,133 | 103,7 | +3,7 |
| 0,1598 | 0,348 | 0,351 | 0,163 | 102,0 | +2,0 |

TABLE 3.

| Sample of raw material, g | Glycyrrhizic acid content | | | Maximum deviation from the mean, % |
|---|---|---|---|---|
| | found | | mean of two determinations, % | |
| | mg | % | | |
| 1,8064 | 0,278 | 4,27 | 4,210 | ±1,42 |
| | 0,270 | 4,15 | | |
| 1,0220 | 0,266 | 4,10 | 4,115 | ±0,37 |
| | 0,270 | 4,13 | | |
| 1,0129 | 0,282 | 4,10 | 4,100 | 0,0 |
| | 0,282 | 4,10 | | |
| 1,1604 | 0,345 | 4,35 | 4,305 | ±1,04 |
| | 0,340 | 4,26 | | |
| 1,3006 | 0,276 | 4,89 | 4,995 | ±2,05 |
| | 0,274 | 5,10 | | |

## EXPERIMENTAL

Analysis of a Thick Extract. A 0.5-g sample (accurately weighted) of the thick extract was dissolved in 25 ml of 50% ethanol. An 18 × 24 cm glass plate with a fixed layer of type L 5/40 μ silica gel was divided into four parallel bands. The first band served as background (blank test) in spectrophotometry, on each of the second and third was deposited 0.12 ml of the solution of the thick extract in the form of a continuous line, and on the fourth a solution of a standard solution of ammonium glycyrrhizate. The plate with the substances deposited on it was dried in the air and chromatographed in the above-mentioned solvent system. After chromatography, the plate was dried in the air at room temperature and was heated in a thermostatted chamber at 95-100°C for 5-10 min after which the second and fourth bands were sprayed with a 1% solution of vanillin in sulfuric acid. On the basis of the spots that appeared, the zone corresponding to glycyrrhizic acid in the third band was marked out. The silica gel with the glycyrrhizic acid was transferred quantitatively to a flask with a ground-in stopper and the acid was eluted with 10 ml of 50% ethanol with continuous shaking for an hour. In precisely the same way, a zone of pure silica gel from the first band was eluted. Then the solutions were centrifuged at 4000 rpm for 20 min, the eluate was carefully transferred to a cell with a pipette, and its optical density at 252 nm was determined.

The optical density of a solution of a standard sample of ammonium glycyrrhizate in 50% ethanol with a concentration of 0.03-0.04 mg/ml was determined in parallel. The percentage of glycyrrhizic acid in the thick extract (x) was calculated from the formula

$$x = \frac{C_{st} \cdot D_x \cdot V_1 \cdot V_3 \cdot 10000}{D_{st} \cdot a \cdot V_2 (100 - h)},$$

where D is the optical density of the solution under investigation; $D_{st}$ is the optical density of the solution of the standard sample; $C_{st}$ is the concentration of the solution of the standard sample, mg/ml; $V_1$ is the volume of eluate, ml; $V_2$ is the volume of the solution under investigation that was deposited on the chromatogram, ml; $V_3$ is the volume of the solution taken for dissolving the weighed sample of thick extract, ml; $a$ is the weight of thick extract, mg; and h is the amount of moisture in the sample of crude extract, %.

The average amount of glycyrrhizic acid in a thick extract was 15.49%.

Analysis of Dry Licorice Powder. A 0.5-g sample (accurately weighed) of dry licorice powder was dissolved in 10 ml of 50% ethanol, and 0.06-ml portions of the solution were deposited on a chromatogram. Then analysis was carried out similarly to the determination of glycyrrhizic acid in the thick licorice extract.

Analysis of Licorice Roots. Licorice root comminuted and passed through a sieve (with 0.5-mm apertures) (1 g) was extracted with 50% ethanol in a Soxhlet apparatus for 8 h. The

extract was evaporated to 25 ml and was deposited on a plate with a fixed layer of silica gel in 0.18-ml portions. Chromatography, elution, and quantitative determination were performed as described above.


## SUMMARY

A spectrophotometric method for determining glycyrrhizic acid in a thick licorice extract, dry licorice powder, and licorice roots after its chromatographic separation from the accompanying substances in a thin layer of sorbent has been proposed. The limit of detection of glycyrrhizic acid is 8 μg/ml. The concentration of glycyrrhizic acid in the thick extracts is ∿15.49%, in the dry powder ∿12%, and in licorice roots ∿4.3%.


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# TRITERPENE GLYCOSIDES OF *Acanthophyllum gypsophiloides*

## V. D-QUINOVOSE IN ACANTHOPHYLLOSIDES B AND C


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As reported previously [1], in a study of the monosaccharide composition of acanthophyllosides B and C by gas–liquid chromatography (GLC) in the form of the trimethylsilyl derivatives, among the peaks belonging to D-xylose, L-arabinose, L-rhamnose, D-fucose, D-galactose, D-glucose, and D-glucuronic acid we found the peak of an unknown compound. It was impossible to determine its qualitative nature in thin-layer and paper chromatograms (TLC and PC) in various systems. By a comparison of various samples of monosaccharides by the GLC method, the unknown sugar has now been identified as D-quinovose.

An investigation of the fractions of individual monosaccharides obtained by separating a hydrolyzate of acanthophyllosides B and C on a column of cellulose has shown that D-quinovose was present in the fractions usually containing L-rhamnose. The complexity of the separation of the monosaccharides is apparently due to the closeness of their $R_f$ values. It is possible to separate D-quinovose distinctly in a mixture with other sugars only by chromatographing the trimethylsilyl derivatives of the methyl glycosides. Where the acetates of the aldononitriles are used in GLC, the peak of D-quinovose is superimposed on the peak of D-fucose [2].

The difficulty in the identification and the separation of D-quinovose and L-rhamnose by the usual methods in natural mixtures has been discussed repeatedly in the literature. Various methods have been proposed for isolating these methylpentoses of similar structure [3-5]. We have attempted to use for this purpose a borate buffer on paper, but we were unable by this method, either, to obtain D-quinovose preparatively in the individual state.

In order to determine the position of the D-quinovose in the carbohydrate chains of acanthophyllosides B and C it was most desirable to start from an analysis of the methylated sugars. From a mixture of the methyl ethers of the monosaccharides, the separation on a column of silica gel and preparative separation on plates we obtained two substances one of which


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