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Preparation and surface-active properties of glycyrrhizic acid and its salts

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Methods are given for the preparation in a pure form of glycyrrhizic acid and its salts. From conductivity and surface tension measurements and unsuccessful solubilisation studies it would appear that micelle formation does not occur in pure solutions of these substances.

JAMES & Stanford (1962) have found from conductance and surface tension measurements that liquorice extracts contain at least two amphipathic substances which form micelles, and which, they suggested were probably glycyrrhizin and gum. Solubilisation by the extracts of benzene, chloroform and hexane was also demonstrated. Glycyrrhizin was isolated from liquorice by Tschirch & Cederberg (1907), but their method gives a poor yield of a coloured product, heavily contaminated with gum, and difficult to purify. No other method of isolation has been reported, but all subsequent workers who have described glycyrrhizin agree that it is a complex salt of glycyrrhizic acid. The major matter of controversy is the identity of the cations; Tschirch & Cederberg (1907) said that glycyrrhizin is a mixed calcium and potassium salt, but Paris (1956) and Pointet-Guillot (1958) have described it as the calcium and ammonium salt, while Tocco-Tocco (1924) and Orsi (1956) claim that it is a mixed potassium and ammonium salt.

In view of the indeterminate nature of the cations of glycyrrhizin and the fact that the surface-active properties would be due to the anion, whose identity is agreed, we have preferred to examine the surface activity of glycyrrhizic acid and some of its salts to determine if this molecule is in fact one of the solubilising constituents of liquorice.

Experimental

Analyses for C, H and N were made by the Department of Chemistry, Welsh College of Advanced Technology, Cardiff, and the Department of Pharmaceutical Chemistry, School of Pharmacy, London.

Potassium was determined volumetrically after incineration, and ammonia distillations were carried out in a Kjeldahl apparatus using octanol as an anti-foam agent. Water contents were determined by drying to constant weight over calcium chloride *in vacuo* at 90°. Spectral absorption between 200–300 mμ was measured with a Uvispek quartz spectrophotometer.

PREPARATION OF MATERIALS

Monoammonium glycyrrhizate was prepared from ammoniated glycyrrhizin (Stafford Allen Ltd.) by a method described by Marsh & Levvy (1956). In preference to their lead salt method, the residue was

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purified by recrystallising four times from 80% ethanol, using decolourising charcoal each time, and twice from 85% ethanol. Yield 2·1%. M.p. 211–217° (decomp.) after turning yellow at 169–170°. $[\alpha]_D^{20}$ + 45·1° (*c* 3·1 in 40% EtOH). Found: C, 54·8; H, 8·3; N, 1·6; NH_3, 1·9; H_2O, 7·8. $C_{42}H_{65}O_{16}N,4H_2O$ requires C, 55·3; H, 8·1; N, 1·5; NH_3, 1·9; H_2O, 7·9. λ_{max} (water) 256 mμ (log ε 4·05), λ_{max} (EtOH) 248 mμ (log ε 4·05). Marsh & Levvy (1956) gave m.p. 212–217° (decomp.), $[\alpha]_D^{20}$ + 46·9° (*c* 1·5 in 40% EtOH), λ_{max} (EtOH) 248 mμ (log ε 4·06). Water content equivalent to $5H_2O$. Onrust, Jansen & Wöstmann (1955) quote a water content equivalent to $4H_2O$.

Tripotassium glycyrrhizate was prepared from monoammonium glycyrrhizate according to the method of Voss, Klein & Sauer (1937). Yield 90% $[\alpha]_D^{20}$ + 41·5° (*c* 4·3 in 40% EtOH), $[\alpha]_D^{20}$ + 46·3° (*c* 4·0 in water). Found: C, 49·4; H, 6·9; K, 11·9; H_2O, 7·15. $C_{42}H_{59}O_{16}K_3$, $4H_2O$ requires C, 50·0; H, 6·7; K, 11·6; H_2O, 7·1 λ_{max} (water) 257 mμ (log ε 4·04). Voss, Klein & Sauer (1937) gave $[\alpha]_D^{19}$ + 44·8° (in water) and water content equivalent to $2H_2O$.

Monopotassium glycyrrhizate was prepared by dissolving tripotassium tetrahydrate (1·3 g) in a mixture of glacial acetic acid (6 ml) and ethanol (4 ml) under reflux. After filtration the solution was allowed to stand for 24 hr. Separation and washing with ethanol gave 0·8 g (62%) of monopotassium glycyrrhizate tetrahydrate as white crystals. $[\alpha]_D^{20}$ + 43·8° (*c* 3·0 in 40% EtOH). Found: C, 54·6; H, 7·3; K, 4·0; H_2O, 7·7. $C_{42}H_{61}O_{16}K$, $4H_2O$ requires C, 54·1; H, 7·45; K, 4·2; H_2O, 7·7. λ_{max} (water) 256–257 mμ (log ε 4·05). Voss, Klein & Sauer (1937) quote a water content equivalent to $2H_2O$.

Glycyrrhizic acid was prepared by suspending monoammonium glycyrrhizate (1 g) in 1% sulphuric acid (10 ml) for 30 min on a hot water-bath. It was important to avoid excessive heating to prevent conversion into glycyrrhetic acid. On cooling, the turbid solution gave a white precipitate which was filtered off and washed with purified water until free of sulphuric acid. Further washing with chloroform gave on drying 0·58 g (58%) of glycyrrhizic acid tetrahydrate. M.p. 203–206°. $[\alpha]_D^{20}$ + 56·4° (*c* 3·1 in EtOH). Found: C, 56·1; H, 7·7; H_2O, 8·0. $C_{42}H_{62}O_{16}$, $4H_2O$ requires C, 56·4; H, 7·9; H_2O, 8·05. λ_{max} (water) 256 mμ (log ε 4·05).

Monosodium glycyrrhizate was supplied by W. H. Ransom Ltd. This material (5·6 g) was recrystallised twice from 80% ethanol, using decolourising charcoal each time, and twice from 85% ethanol to give 2·5 g (45%) of monosodium glycyrrhizate tetrahydrate as white crystals. $[\alpha]_D^{20}$ + 45·0 (*c* 3·1 in 40% EtOH). Found: C, 55·25; H, 7·9; H_2O, 7·9. $C_{42}H_{61}O_{16}Na$, $4H_2O$ requires C, 55·0; H, 7·6; H_2O, 7·9. λ_{max} (water) 256–257 mμ (log ε 4·05).

CONDUCTANCE MEASUREMENTS

The water used for the preparation of solutions and for conductance measurements was prepared by passing distilled water through a bed of

mixed ion-exchange resins and then allowing it to equilibrate with the atmosphere for 24 hr. Its specific conductance, at 25°, varied between $1{\cdot}21$–$1{\cdot}36 \times 10^{-6}$ mhos cm^{-1}.

The conductances of aqueous dilutions of glycyrrhizic acid and its salts were measured at $25 \pm 0{\cdot}1°$, using a Pye conductance bridge. Plots of equivalent conductance against the square root of molarity for glycyrrhizic acid and its monoammonium and tripotassium salts, are shown in Fig. 1. Since the conductance curves for the mono-substituted salts were nearly identical only one of these has been included.

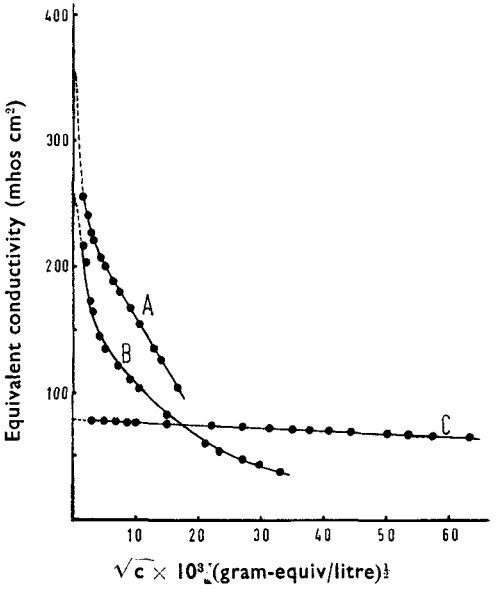

FIG. 1. Variation of equivalent conductivity of glycyrrhizic acid and its salts with concentration. A, Glycyrrhizic acid. B, Monoammonium glycyrrhizate. C, Tripotassium glycyrrhizate.

SURFACE TENSION MEASUREMENTS

A static method (Wilhelmy plate) was used for the measurement of surface tensions. The apparatus employed has been described by Robins & Thomas (1963). Measurements were made on aqueous dilutions of glycyrrhizic acid and its salts and surface tensions plotted against molarity: the graphs are shown in Fig. 2.

The surface tensions of solutions of glycyrrhizic acid and its salts did not change with time.

SOLUBILISATION EXPERIMENTS

Aqueous solutions of various concentrations of glycyrrhizic acid and its salts were shaken for 6 hr at 25° with dimethyl yellow. Insoluble material was then filtered off and the extinction of the filtrate determined using a Spekker absorptiometer. No significant increase in absorption

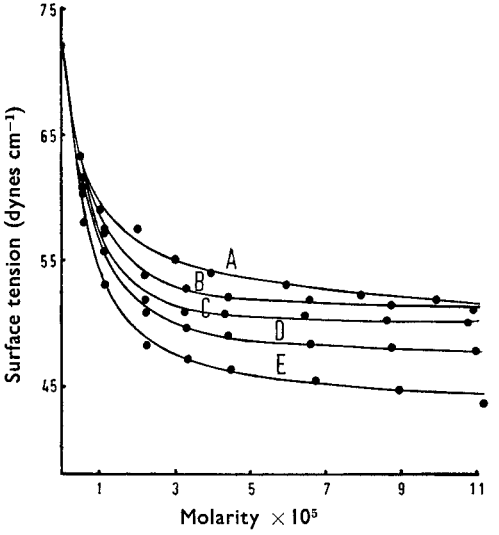

FIG. 2. Variation of surface tension of glycyrrhizic acid and its salts with concentration. A, Tripotassium glycyrrhizate. B, Monosodium glycyrrhizate. C, Monopotassium glycyrrhizate. D, Monoammonium glycyrrhizate. E, Glycyrrhizic acid.

was observed over that of the control with water only. To confirm this result, chloroform, which has been shown to be solubilised by liquorice extracts (James & Stanford, 1962) was examined. Various quantities were shaken with 0·01M tripotassium glycyrrhizate, and optical densities measured as before. The extinction rose rapidly as soon as the aqueous solubility of chloroform was exceeded, indicating that emulsification and not solubilisation, had taken place.

Discussion

The many methods that have been described for the preparation of glycyrrhizic acid and its salts have been reviewed recently (Muravev & Ponomarev, 1962; Gilbert, 1963). After critically examining most of these, we are in agreement with Brieskorn & Mahran (1960) that much of the work is difficult to reproduce. The methods of preparation we have used were those we considered to be the most satisfactory. Our main difficulty was to establish the purity of our product, the usual criterion, namely melting-point, was of little value, since only that of the acid and that of the monoammonium salt were sufficiently low, and these are known to extend over several degrees. Other reported physical properties are both few and variable and when quoted, the purity of the material has not been proved. For this reason we quote a full analysis for each compound even though none of them is new. To prepare solutions of known molarity, it was necessary to know the quantity of water of crystallisation and moisture determinations were made because of the lack of agreement amongst the published figures, $4H_2O$ (Onrust,

Jansen & Wöstmann, 1955) and $5H_2O$ (Marsh & Levvy, 1956) for monoammonium glycyrrhizate, and $2H_2O$ (Voss, Klein & Sauer, 1937) for the monopotassium and tripotassium salts. The presence of $4H_2O$ was confirmed by the elemental analysis, and was the same for all the compounds; this was anticipated since the water would be expected to be bound to the anion. A report (Tschirch & Cederberg, 1907) that glycyrrhizic acid salts form yellow solutions in aqueous alkali was tested by observing the effect of alkali on the electronic spectra. Not even 5N sodium hydroxide could produce a bathochromic shift and so it was assumed that this early observation was due to impurities in the sample.

Fig. 2 shows that the surface tension is greater when the acid is neutralised with strong basic cations. The curves are typical of amphipathic substances, and their shape could suggest micelle formation. This was considered unlikely however since the surface tension continued to decrease after the initial sharp fall. Similar curves were obtained with the saponins by Ruyssen & Loos (1947) who confirmed by several other methods that the systems they studied did not form micelles.

The conductance curve for glycyrrhizic acid is typical of that for a weak electrolyte and that for tripotassium glycyrrhizate typical of a strong electrolyte. For the mono-substituted salts, the unneutralised carbonyl groups behave as weak electrolytes, while the carboxyl group involved in salt formation is completely ionised, and, as expected, the curves for these salts lay between those for glycyrrhizic acid and the tripotassium salt (Fig. 1). Extrapolation of the straight line for tripotassium glycyrrhizate gave a limiting equivalent conductance of 79·5 mhos cm^2, equivalent to a mobility of 18 mhos cm^2 $equiv^{-1}$ for the glycyrrhizate ion. This agrees favourably with the values of 15 and 16 mhos cm^2 $equiv^{-1}$ respectively quoted for the similar sapoalbin and senegin ions by Ruyssen & Loos (1947). Accurate extrapolation of the conductance curves of the other compounds was not feasible, but the approximate limiting conductances obtained by this means agreed with those calculated assuming an anionic mobility of 18 mhos cm^2 $equiv^{-1}$. There is therefore no sharp decrease in the slopes of these curves, and these compounds, like the tripotassium salt, are therefore molecularly dispersed electrolytes. The solubilisation experiments support this conclusion.

The glycyrrhizin content of liquorice root differs from plant to plant but is usually within the range 7·0–10·0% (Nieman, 1957) and an aqueous extract containing 7·5% has been prepared by James & Stanford (1962). In this study, aqueous solutions of tripotassium glycyrrhizate could be prepared up to a concentration of 4% but heating was necessary to aid solution. For glycyrrhizic acid and monoammonium glycyrrhizate 0·03 and 0·5% aqueous solutions, respectively, could be prepared only by vigorous mixing at 80°, and even much weaker solutions required a little heat to aid solution. Our observations suggest that glycyrrhizin behaves differently in liquorice extracts than in aqueous solution. It is possible that other constituents in liquorice may either promote micelle formation, or combine with glycyrrhizin to form compound micelles.

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