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CONSTITUENTS OF LOCAL PLANTS—VI[1]

LIQUORIC ACID, A NEW TRITERPENOID FROM THE ROOTS OF *GLYCYRRHIZA GLABRA* L.

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Abstract—A new pentacyclic triterpenoid, liquoric acid, has been isolated from the roots of *Glycyrrhiza glabra* L. as the methyl ester. Its properties are consistent with the formulation as a glycyrrhetic acid derivative containing an extra oxygen atom as an ethereal bridge, probably between the 16- and 21-positions.

ROOTS of liquorice, *Glycyrrhiza glabra* L. are the source of the pentacyclic triterpenoid glycyrrhetic acid, the constitution[2] and stereochemistry[3] of which are now established. Our interest was directed to the minor constituents which result together with glycyrrhetic acid from mineral acid hydrolysis of the liquorice saponin mixture. To the best of our knowledge, the only report on such products is that of Beaton and Spring.[4] These workers isolated a very small amount of the epimer, 18α-glycyrrhetic acid and did not preclude the possibility that it might be an artifact resulting from the acid-induced isomerization of glycyrrhetic acid during saponin hydrolysis. Moreover, the same authors reported on another minor constituent, glabric acid, which possesses the same fundamental constitution as 18α-glycyrrhetic acid but differs from it in being dihydroxylic.

The processing of a large amount of liquorice root for the isolation of the triterpenoids from the saponin hydrolysate led to the removal of large amounts of glycyrrhetic acid as acetate. The mother liquors were further processed, including a stage of methylation, and subjected to repeated chromatography (Experimental) to afford two pure crystalline products "A" and "B" which were previously deacetylated.

The first "A", m.p. 239–241°, $[\alpha]_D$ +7·88° ($CHCl_3$) gives a positive Liebermann-Burchardt reaction for triterpenoids and, because of its isolation in extremely limited amounts, only speculative information regarding its nature could be drawn from qualitative tests. Its behaviour on thin layer chromatography indicates that it is monohydroxylic with no reactive ketonic function or an aldehyde group.[5] It is unsaturated (TNM) and absorbs in the UV region below 210 mμ. The IR spectrum reveals an hydroxyl absorption near 3300 cm^{-1} and an ester C=O absorption at

[1] Part V.: M. B. E. Fayez, S. A. R. Negm and A. Sharaf, *Planta Medica* **11**, 439 (1963).
[2] For pertinent literature cf. C. Djerassi and C. M. Foltz, *J. Amer. Chem. Soc.* **76**, 4085 (1954); O. Jeger, *Fortsch. Chem. Org. Naturstoffe* **7**, 45 (1950) and Refs cited therein.
[3] J. M. Beaton and F. S. Spring, *J. Chem. Soc.* 3126 (1955).
[4] J. M. Beaton and F. S. Spring *J. Chem. Soc.* 2417 (1956).
[5] Details of this method for detection of ketonic functions by the use of 2,4-dinitrophenylhydrazine on thin layers and the systems used for the differentiation of triterpenoid acid types according to hydroxyl content are to be published in *J. Chromatog.*

1730 cm^{-1}. Further, the spectrum contains peaks at 1380 and 1360 cm^{-1}, which may be an indication of a pentacyclic triterpenoid constitution of the oleanane type in accordance with a recent publication by Snatzke *et al.*[6] The same authors also state that the tetracyclic triterpenoid acids or their esters show only one strong absorption in this region in addition to one peak in the 1330–1250 cm^{-1} region. In the latter region, product "A" exhibits one appreciable peak near 1310 cm^{-1} while an oleanolic acid type of constitution would be expected to exhibit three bands of increasing intensity in the same region.[6] In the absence of more concrete evidence, the speculative conclusions based on spectral and other data indicate that product "A" is the methyl ester of a new acid belonging to either the β-amyrin or tetracyclic triterpenoid acid[7] series—the latter being less probable on account of the high m.p. observed. The evidence also excludes all the known related natural triterpenoid acid derivatives as well as the structural variant, methyl 11-desoxoglycyrrhetate, by direct comparison.

The second crystalline product "B" isolated from the methylated mother liquors of glycyrrhetic acid has m.p. 260–263° and $[\alpha]_D$ +155·2° ($CHCl_3$). It gives a pink colouration in the Liebermann-Burchardt reaction and no colour with tetranitromethane. Elemental analysis is in accord with either $C_{31}H_{46}O_5$ or $C_{31}H_{48}O_5$ but the mass spectral measurements support the former formulation. A consideration of the properties of this product and its origin shows that it is a new triterpenoid for which the name "liquoric acid", isolated as the methyl ester, is proposed. The behaviour of this substance on the chromatoplates indicates that it can not possibly contain more than one hydroxyl which is similarly positioned as in methyl glycyrrhetate. The relationship to the latter derivative was inferred from the absorption spectra. In UV light methyl liquorate exhibits maximal absorption at 247 mμ ($\varepsilon = 10750$) indicating an α,β-unsaturated ketone chromophore which is also evidenced in the IR spectrum by a strong C=O peak at 1665 cm^{-1} and a C=C peak near 1625 cm^{-1}. In addition to the hydroxyl absorption, the spectrum contains a carbonyl absorption at 1740 cm^{-1} which must be attributed to a carbomethoxyl and this is supported by the micro-saponification of methyl liquorate. Although free liquoric acid has not been obtained by preparative means, the product of saponification occupies a position on the chromatoplates close to that of glycyrrhetic acid and yet quite remote from that of the methyl ester. Methyl liquorate acetate, m.p. 256–258°, $[\alpha]_D$ +166·6°, prepared by the usual as well as under forcing conditions, also occupies a position corresponding to that of methyl glycyrrhetate acetate on the chromatoplates. Its IR absorption spectrum contains no evidence of hydroxyl absorption.

As to the fundamental skeleton, the IR absorption spectrum shows the presence of two bands in the "A-region" near 1390 and 1370 cm^{-1} and four bands near 1330, 1316, 1280 and 1260 cm^{-1} in the "B-region", which is not consistent with either oleanolic or ursolic acid types of constitution. Such a four-band absorption pattern in the "B-region" is, however, characteristic of glycyrrhetic acid and its methyl ester, as observed and as reported,[6] and furthers a structural affiliation between methyl liquorate and methyl glycyrrhetate.

The data given so far suggest the presence of hydroxyl, α,β-unsaturated ketone

[6] G. Snatzke, F. Lampert and R. Tschesche, *Tetrahedron* **18**, 1417 (1962).

[7] Several new tetracyclic acids have recently been isolated from a number of *Glycyrrhiza* species, N. P. Kir'yalov and T. N. Naugol'naya, *Zh. Obshch. Khim.* **33**, 694, 697, 700 (1963).

and carboxyl functions in the pentacyclic oleanane skeleton of liquoric acid. Regarding the position of these groups, it may be assumed—considering the probable common biogenetic origin of liquoric and glycyrrhetic acids—that one oxygen is present as the 3β-hydroxyl, another as a carbonyl in a 12-en-11-one system and two in a carboxyl group attached, by analogy, to the 20-position. Thus four of the five oxygens in the formula $C_{31}H_{46}O_5$ are accounted for. The fifth oxygen can not be a second hydroxyl group since the position of methyl liquorate and liquoric acid on the chromatoplates which correspond to methyl glycyrrhetate and glycyrrhetic acid are decidedly remote from the positions of the corresponding dihydroxylated products. The molecular formula could only accommodate a second hydroxyl if an isolated double bond were also present which is not the case (TNM). In addition, evidence from the mass spectra, *vide infra*, clearly precludes this possibility. The fifth oxygen as a nuclear carbonyl does not find support in the IR spectrum where a corresponding band in the 1720–1700 cm^{-1} region is not present. The spectrum of methyl 3-oxo-glycyrrhetate, incidentally, indicates the presence of such a band near 1718 cm^{-1} among the total carbonyl make-up. Moreover, the spectra of both methyl liquorate and methyl glycyrrhetate are identical over the entire carbonyl region of absorption. Methyl liquorate does not react with 2,4-dinitrophenylhydrazine, which precludes the possibility of an aldehyde or a reactive carbonyl group.

The mass spectrum of methyl liquorate affords additional information regarding its constitution as a glycyrrhetic acid congener. Apart from the molecular ion (*m/e* 498), the spectrum contains a peak due to the dehydrated form (*m/e* 480), both corresponding in intensity to those due to methyl glycyrrhetate, but there is no trace of a M-36 peak which excludes the possibility of a dihydroxylic constitution. The spectra of methyl liquorate and methyl glycyrrhetate both exhibit ions of poor abundance due to the loss of one methyl and ions of appreciable intensity due to the loss of both water and methyl. They differ, however, in that while the spectrum of methyl glycyrrhetate exhibits an ion corresponding to the loss of 59 mass units (CO_2CH_3), which must be a rare event,[8] and a dehydrated form thereof at *m/e* 407, the spectrum of methyl liquorate is devoid of any such peaks. This indicates a difference in the environment surrounding the carbomethoxy groupings in both molecules.

The most characteristic fragmentation of diagnostic value of 12-unsaturated oleananes has been outlined by Budzikiewicz *et al.*[8] and takes place across ring C by a retro-diels-Alder reaction. This has been observed to lead to a fragment at *m/e* 290 in the spectrum of methyl liquorate and a corresponding fragment (I) at *m/e* 276 of about the same abundance in the spectrum of methyl glycyrrhetate. This difference of 14 mass units suggests the location of the remaining fifth oxygen (as ketone or ether bridge) in or around rings D and E or on C-27. It is known[8] that such species (as I) usually suffer further decomposition by loss of the angular C-17 substituent if the latter is a carbomethoxy, a lactone, or a CH_2OAc leading to an ion of considerable abundance exceeding that of the parent ion (I). No such ions as *m/e* (290–59) or even due to the loss of the C-17 methyl are observed in the spectrum of methyl liquorate, which would unequivocally exclude the possibility of C-17-assignment for the carbomethoxy. The presence of an 11-carbonyl, as in glycyrrhetic acid and its congeners, is also reportedly[8] known to lead to yet another and specific fragmentational

[8] H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.* **85**, 3688 (1963).

behaviour which takes place across ring B. This has given rise to the strongest peak in the spectrum of methyl glycyrrhetate at *m/e* 317 and also to the most abundant ion, 14 mass units higher, in the spectrum of methyl liquorate at *m/e* 331. This provides further proof that the additional oxygen function must be on the "right" side of the molecule and that methyl liquorate does indeed contain a 12-en-11-one moiety. The genesis of this species (II), as proposed by Budzikiewicz *et al.*[8] is evidenced in the spectrum of methyl glycyrrhetate by a metastable ion at *m/e* 208; calculated 207·6 for *m/e* 484 → *m/e* 317.

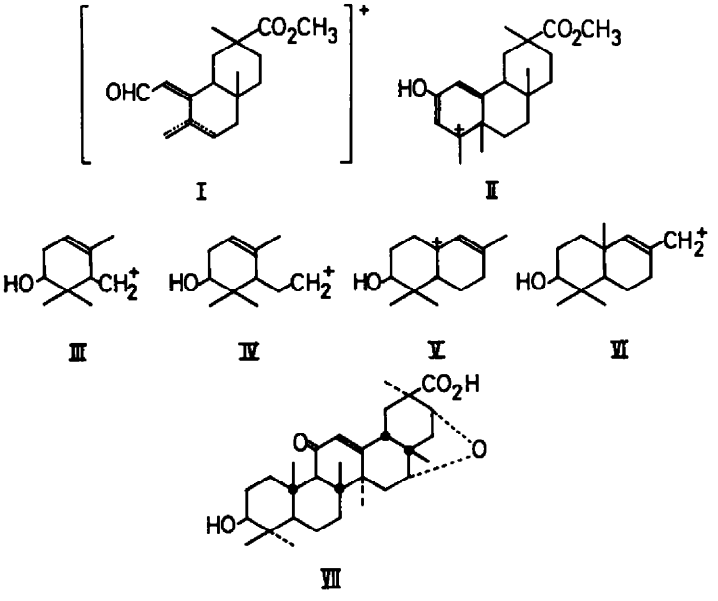
The fission across ring B leads to two other fragmentation products containing an intact ring A and remnants of B. The first is represented by the peak (*m/e* 135; about 75% of the intensity of the base peak) in the spectra of both methyl glycyrrhetate and methyl liquorate. It is not unlikely that it possesses the constitution III or one akin to it, detected only in the dehydrated form. This fragment has probably also existed in the published spectra of some other pentacyclic triterpenoids[8] but does not seem to have been accounted for. The second fragment appears as a peak with appreciable intensity at *m/e* 149 in both spectra and probably represents the "remaining" portion of the molecule corresponding to fragment II. It is represented by IV and is also only detected in the dehydrated form. These two species are characteristic of the fragmentation process across ring B induced by the presence of an 11-keto group. Another fragment of marked abundance is observed in both spectra at *m/e* 175. To account for its origin, it may be assumed that the "neutral" fragment constituting rings A and B remaining after fission across ring C[8] and subject to further electron bombardment leads to the loss of its angular methyl (V). The mass number given is for the dehydrated species and a hydroxylated satellite of *m/e* 193 can also be detected in appreciable amounts. Another fragment with appreciable intensity has also been detected in the mass spectra of both compounds at *m/e* 189. Its formation is envisaged to be the outcome of another fission across ring C and may be represented by VI detected only in the dehydrated form. Fragments of similar constitution have also been reported[8] from several ring C unsaturated oleananes with no substitution in or around rings A and B. The presence of these four fragments (III–VI)[9] in both spectra provides additional evidence that the constitutions of both glycyrrhetic and liquoric acids are identical in and around rings A and B.

The possibility that the first oxygen might be a nuclear ketone located at one of the only positions C-15, C-16, C-19, C-21 and C-22—after the exclusion of the aldehyde possibility—is not supported by the mass spectral evidence. The implications of ketones situated in such positions have been discussed in detail by Budzikiewicz *et al.*[8] The data afforded by cicular dichroism provides additional support, and perhaps conclusive evidence, that the carbonyl chromophoric content in both methyl liquorate and methyl glycyrrhetate is identical. The circular dichrograms are practically identical and that of methyl liquorate does not contain any appreciable effect at about 300 mμ. This can safely be taken as proof that methyl liquorate contains no second carbonyl function unless the Cotten effect is extremely small.

The fifth oxygen in methyl liquorate must, therefore, be an ethereal bridge, but in view of the absence of direct evidence, its position can only be speculative based on analogy with deviation from methyl glycyrrhetate. The occurrence of ethereal

[9] Experimental work is currently in progress to verify the genesis of these species.

linkages in the "right" side of the molecule in pentacyclic triterpenoids with a Δ^{12}-double bond has so far been encountered in only two cases, namely aescigenin[10] and barringtogenol-D[11] where the ether engages the 16- and 21-positions as inferred from the modified properties of the functional groups located in this vicinity. The properties of methyl liquorate, differ from those of methyl glycyrrhetate and may well be due to the different environment. Thus, it will be recalled, the mass spectra of both products exhibit a marked difference with respect to the losses involving the carbomethoxy grouping attached to C-20. It is not unlikely, therefore, that the bridge has



I II III IV V VI VII

its origin in one of the adjacent positions, namely C-19 or C-21. In view of the existence of such ethereal bridges in this area, it may provisionally be assumed that the bridge in liquoric acid is likewise located between C-21 and C-16 (VII). Inspection of a Dreiding model with such a constitution shows that the oxygen bridge has only a negligible effect on the angle between the C=C and the C=O double bonds on account of the relative flexibility of ring C.[12] This would not, therefore, be expected to produce any marked effect on the circular dichroism.

With regard to the stereochemistry of liquoric acid, it may be assumed that it possesses all the configurations at the asymmetric centres of triterpenoids of this type. This implies a 3β-configuration for the hydroxyl and most probably also a β-configuration and, therefore, axial conformation of the carboxyl. The latter assignment finds support in the following evidence. First, the mass spectrum of methyl liquorate exhibits, as does that of methyl glycyrrhetate, the fragment II, *m/e* 331 and 317 respectively, with somewhat more abundance than fragment I, *m/e* 290 and 276 respectively, as might be expected[8] for such constituted 18β-derivatives. With the 18α-epimers the relative intensities would, however, be expected[8] to change strongly

[10] G. Cainelli, A. Melera, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **40**, 2390 (1957).

[11] S. K. Chakraborti and A. K. Barua, *Experientia* **18**, 66 (1962).

[12] In this connection it should be noted that the 25–26 dimethyl interaction affects this angle more, cf. G. Snatzke, *Tetrahedron* **21**, 421 (1965).

in favour of the first fragment.[13] Second, the α,β-unsaturated ketone absorption in the UV light for glycyrrhetic acid and its derivatives (at 248 mμ) is[4] different from the corresponding 18α-series (242–244 mμ). This difference helps to support the 18β-configuration in liquoric acid which exhibits maximal absorption at 247 mμ. As a corollary, the carboxyl group would be axially bound (β), an assignment which is inferred from the observation that the carbomethoxy group in methyl liquorate is not easily saponified. In this respect it corresponds with a similar group in methyl glycyrrhetate and is in contrast[3] to the easily saponifiable group in methyl 18α-glycyrrhetate. The constitution and stereochemistry of liquoric acid, isolated as methyl ester, may, therefore, tentatively and pending further work be represented by VII.

EXPERIMENTAL

UV spectra. A Carl Zeiss PMQII spectrophotometer was used for the determination of the UV spectra, solvent being spectroscopic EtOH.

Optical rotations. Values of $[\alpha]_D$ were determined using $CHCl_3$ as solvent in 1 dm tubes.

IR spectra. The IR spectra were determined on a Perkin-Elmer "Infracord 137B" instrument and taken in Nujol and in $CHCl_3$ solutions.

Mass spectra. The measurements were made on an Atlas CH 4 spectrometer with an ion source (type TO 4 with vacuum lock) temp. of 200°.

Circular dichroism. The measurements were taken on a Roussel-Jouan dichrograph in dioxan solution at 20°, 2 cm path length.

The minor co-constituents of glycyrrhetic acid

Finely powdered liquorice root (100 Kg) processed as described by Beaton and Spring[4] for the isolation of glycyrrhetic acid afforded a total yield of about 0·34% of that acid, as acetate. A portion (50 g) of the dried mother liquors, corresponding to about half the amount, was dissolved in $CHCl_3$ and methylated with diazomethane ethereal solution. The resulting material was then chromatographed on alumina (1500 g) and the column eluted progressively with pet. ether, benzene, ether, acetone and alcohol alone and in mixtures of increasing polarity. A pet. ether–benzene mixture (1:1) removed a further amount of glycyrrhetic acid as the methyl ester acetate m.p. 295–298°, $[\alpha]_D$ +146·3°; reported[14] m.p. 299–301°, $[\alpha]_D$ +145°. The combined mother liquors from this band were saponified by refluxing with 5% methanolic NaOH for 2 hr and the resulting material (2 g) again chromatographed on alumina (360 g). Two principal products were thus obtained: "A" (19·5 mg) eluted with hexane–benzene mixture (1:2) and "B" or *methyl liquorate* (90·3 mg) eluted with 5% MeOH in benzene.

Product "A". This substance, obtained as fine needles from $CHCl_3$–MeOH, m.p. 239–241°, $[\alpha]_D$ +7·88°, developed in the Liebermann-Burchardt test a violet colour which changed to blue and gave a yellow colouration with the tetranitromethane–$CHCl_3$ reagent. In the UV light it exhibited maximal (stray light) absorption at 206 mμ. The material was indifferent to the action of diazomethane and did not react with 2,4-dinitrophenylhydrazine when examined on thin layers of silica gel followed by development. On the chromatoplates (silica gel G, E. Merck), developed with benzene–acetone (18:2), product "A" occupied a position (R_f 0·72) comparable to that of several monohydroxylic-non-ketonic acid esters and developed a pale blue colour (yellowish green UV fluorescence) upon spraying with chlorosulphonic acid–acetic acid mixture (1:2).

Methyl liquorate. This was obtained as long prismatic plates from $CHCl_3$–MeOH, m.p. 260–263°, $[\alpha]_D$ +155·2°, and gave a pink coloration in the Liebermann-Burchardt reaction and no colour with tetranitromethane. It exhibited maximal absorption in the UV light at 247 mμ (ε = 10750). (Found: C,74·89; H, 9·87%; mol. wt. 498 by mass spectroscopy. $C_{31}H_{46}O_5$ requires: C, 74·36; H, 9·66% and $C_{31}H_{46}O_5$ requires: C, 74·36; H, 9·30%; mol. wt. 498.) The substance was recovered unchanged after treatment with diazomethane and did not react with 2,4-dinitrophenyhydrazine as revealed by

[13] Provided that the same method of introduction into the ion source is used.

[14] L. Ruzicka and H. Leuenberger, *Helv. Chim. Acta* **19**, 1402 (1936).

application of the test on silica gel chromatoplates followed by development. This method[8] worked successfully with all the products containing reactive carbonyls. CD (λ, $\Delta\varepsilon$): 396(0), 387(+0·03), 382(θ), 373(−0·09), 356(−0·28), 341(−0·28), 328(−0·13), 315(θ), positive at shorter wavelengths (c = 1·19 g/l). CD of methyl glycyrrhetate cf. Witz *et al.*[15]

Acetylation. A solution of methyl liquorate (12 mg) in pyridine (0·5 ml) was treated with 1 ml acetic anhydride at room temp. The resulting product was crystallized from methylene chloride–EtOH to give plates, m.p. 256–258°, $[\alpha]_D$ +166·6°. The same product resulted from a treatment of methyl liquorate, or its acetate, with acetic anhydride containing some anhydrous sodium acetate or toluene-*p*-sulphonic acid under reflux for 2 hr.

A quantity (2 mg) of methyl liquorate was saponified[8] by refluxing with 5% methanolic NaOH for 2 hr and, after the usual work-up, the product was inspected on a silica gel G chromatoplate using a system of benzene–acetone (13:2) for development and the chlorosulphonic acid–acetic acid mixture as spray reagent. A spot due to the free liquoric acid (R_f 0·33) was positioned close to that due to glycyrrhetic acid (R_f 0·36). On the same plate methyl liquorate, unchanged ester, had R_f 0·45, methyl glycyrrhetate 0·50, methyl liquorate acetate 0·75 and methyl glycyrrhetate acetate 0·75. When the saponification of methyl liquorate was performed on a larger amount of material, under the conditions given above,[8] most of the methyl ester was recovered unchanged.

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[15] P. Witz, H. Hermann, J.-M. Lehn and G. Ourisson, *Bull. Soc. Chim. Fr.* 1101 (1963).