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Naturally Occurring Lactones and Lactames

I. The Absolute Configuration of Ranunculin, Lichesterinic Acid, and Some Lactones Related to Lichesterinic Acid

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Nuclear magnetic resonance spectra have confirmed the provisional structure of ranunculin. Circular dichroism data allowed the assignment of the configuration of its aglucone to be 4*S*. As a result of the circular dichroism work, it was also possible to allocate configurations to the following lichen lactones: (*S*)-(−)-lichesterinic acid, (3*R*,4*S*)-(−)-protolichesterinic acid, (3*S*,4*S*)-(−)-alloprotolichesterinic acid, and (2*R*,3*S*,4*S*)-nephromopsic acid.

Protoanemonin, 2,4-pentadien-4-olide, obtained from many plants of the family Ranunculaceae, is derived from a glucoside, ranunculin (*1*), first isolated by Hill and van Heyningen.[1] As found by these authors, ranunculin released D-glucose and protoanemonin on warming in sodium acetate solution. It was shown to contain a lactone group and stated to be stable both as a solid and in aqueous solution, relatively stable in acid solution, but rapidly broken down in the presence of alkali, which induces β-elimination. The authors pointed out the analogy between the breakdown of ranunculin and that of the glycoside picrocrocin [2] and put forward a provisional structure (*1*) for ranunculin based upon its stability in acid and the formation of protoanemonin. This structure has been found to be in agreement with the ultraviolet and infrared spectra of ranunculin.[3] In regard to optical rotation, IR spectrum, and action of β-glucosidase, the glucoside has been proposed to be the β-anomer.[4]

Recently, Benn and Yelland [5] have determined the configuration of the aglucone to be 4*S*. Independently, we have reached the same conclusion from circular dichroism data and we have, furthermore, confirmed the provisional structure for ranunculin (*1*). As a result of the circular dichroism work, it is also possible to determine the absolute configuration of (−)-lichesterinic acid (*5*) and other lichen lactones.

Structure. The ranunculin used was isolated from *Pulsatilla vulgaris* Mill., thus representing a new source of ranunculin. The IR and UV spectra were consistent with the above findings, and from the NMR spectrum (Fig. 1) it can be further substantiated that ranunculin is a glucoside of 5-hydroxy-2-2-penten-4-olide. The low field lines appear in three distinct groups each representing one proton. Apparently an AMX system is present, since two of the groups are constituted of well-separated symmetrical quartets, centred upon the chemical shift positions of the two nuclei. The hydrogen on C-2 resonates at $\delta=6.28$ ppm ($J_{2,4}=2.0$ cps; $J_{2,3}=6.0$ cps) and the hydrogen on C-3 at $\delta=7.83$ ppm ($J_{3,4}=1.5$ cps; $J_{2,3}=6.0$ cps). The signal from the hydrogen at C-4 is distorted by the C-5 protons and appears as a multiplet at $\delta=5.35$ ppm and the splitting shows the requisite values for 2,4 and 3,4 CH coupling. The presence in the spectrum of the ABX system made up of the signals from the protons on C-4 and C-5 is not easily detected due to the signals of the glucose moiety.

Acid or alkaline hydrolysis destroys the aglucone, but Hellström [4] has found that ranunculin is hydrolyzed by emulsin to yield, judged from paper chromatography and optical rotation, an optically active aglucone. Repetition of this experiment results in the isolation of the genuine aglucone giving an NMR spectrum (Fig. 2) in which besides the AMX part the two methylene protons and the HO-proton at C-6 is recognized at $\delta=3.78$ ppm and $\delta=2.00$ ppm, respectively.

Concerning the glucose moiety, the doublet occurring at $\delta=4.25$ ppm ($J_{1',2'}=7.0$ cps) in Fig. 1 (b) is assigned to the anomeric hydrogen. The chemical shift and the presence of an 1,2-diaxial coupling is in agreement with the pro-

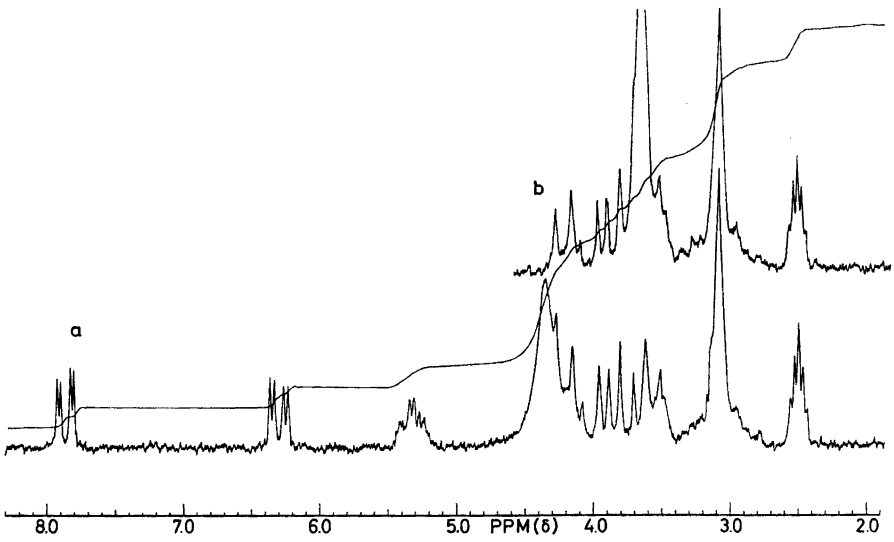


Fig. 1. NMR spectrum at 60 Mc/s of (a) ranunculin in dimethyl sulfoxide-d_6 with tetramethylsilane as reference. (b) represents the signals detected after the addition of deuterium oxide to the solution.

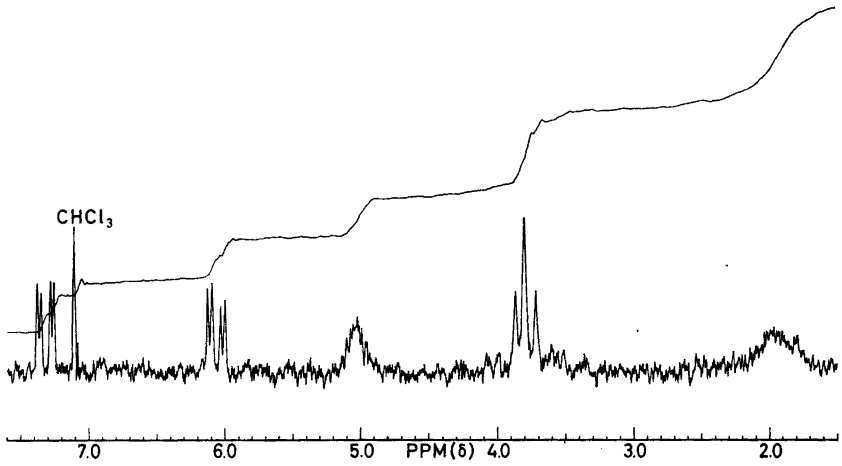


Fig. 2. NMR spectrum of (*S*)-4-hydroxymethyl-2-buten-4-olide in chloroform-*d*.

posal of ranunculin as a β-D-glucoside. This assignment is verified by recording the spectrum in a solution of *ca.* 40 % deuterium bromide in deuterium oxide, in which the doublet at δ ~4.8 ppm * gradually disappears and after 4 days of standing is replaced by signals at δ ~6.5 ppm * ($J_{1',2'}=3.5$ cps) and δ ~5.0 ppm * ($J_{1',2'}=7.5$ cps) indicative of the presence of an equilibrated mixture of α- and β-D-glucopyranose.

The configuration of the anomeric monosaccharide methyl glycosides have been correlated by oxidation with periodate.[6,7] Thus in the D-series all the methyl β-aldohexopyranosides through cleavage of the 2,3,4-triol group yield a common aldehyde, which has a large negative molecular rotation ($[M]_D$ −198°), like the corresponding α-glycosides all yield an aldehyde, which differs in configuration and possesses a large positive rotation. Ranunculin assumes on this oxidation already after 40 min a constant molecular rotation ($[M]_D$ −314°). If one from this value subtracts the molecular rotation of the aglucone ($[M]_D$ −160°), the negative molecular rotation is of the same order of magnitude as found for methyl β-D-aldohexopyranosides. Ranunculin consumes by periodate oxidation 2.3 moles of periodate and no formaldehyde is formed indicating the presence of a pyranoid ring.

NMR measurements of the anomeric proton signals cannot be completely definitive, before it is possible to assign all the signals recorded to the respective protons, because conformations C1, 1B, and B2 [8,9] of the β-anomers have the anomeric proton axially oriented and at a 180° dihedral angle with the proton at C-2′; but due to the rotational differences between aglucone and glucose moiety, it seems reasonable to assume a C1 conformation.

* These values are to be considered with some reservation. They are calculated relative to the water signal ($\delta=5.0$ ppm) in a spectrum of ranunculin in deuterium oxide, although the chemical shifts in two spectra of ranunculin in deuterium oxide and in deuterium bromide/deuterium oxide are not completely identical.

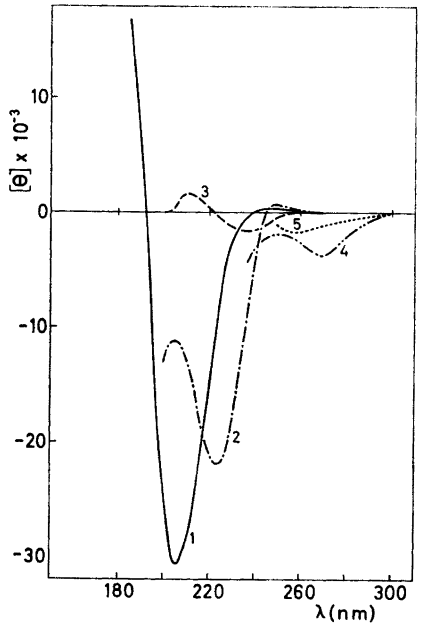


Fig. 3. Circular dichroism curves of (*1*), (*2*), (*3*), (*4*), and (*5*); all in methanol.

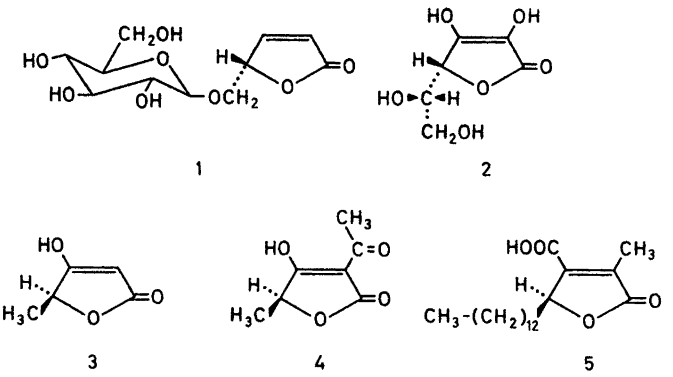
Configuration. The Cotton effects of some α,β-unsaturated carboxylic acids have been examined by Weiss and Ziffer.[10] They focused their attention on the weak $n \rightarrow \pi^*$ transition of the conjugated carbonyl group occurring at about 250 nm. In the case of shikimic and *epi*-shikimic acid they observed that inversion of the asymmetric centre next to the double bond inverts the sign of the Cotton effect, and the sign of the Cotton effect of four α,β-unsaturated acids were consistent with their configuration.

This observation has been extrapolated to α,β-unsaturated γ-lactones [11–13] and the sign of the Cotton effect apparently permits assignment of the correct configuration to the asymmetric centre next to the double bond.

The compounds here investigated were ranunculin (*1*), the tetraacetyl derivative of L-ascorbic acid (*2*), (*S*)-γ-methyltetronic acid [14] (*3*), (*S*)-α-acetyl-γ-methyltetronic acid [14] (*4*), and (—)-lichesterinic acid [15] (*5*). All showed circular dichroism in the range of 240—270 nm apparently corresponding to weak absorption bands in this spectral range and formerly ascribed to $n \rightarrow \pi^*$ transitions of the conjugated carbonyl group [10] (Fig. 3).

Ranunculin (*1*) and the tetraacetyl derivative of L-ascorbic acid (*2*) give positive circular dichroism indicating an identical configuration at C-4. Ranunculin then is assigned the 4*S*-configuration.

Furthermore, (*S*)-γ-methyltetronic acid (*3*) and (*S*)-α-acetyl-(—)-methyltetronic acid (*4*) exhibit negative Cotton effects. The long-wavelength position of the Cotton effect in (*4*) is attributed to its more highly conjugated system. (—)-Lichesterinic acid (*5*), which is a naturally occurring lactone, also exhibits



a negative Cotton effect, strongly suggesting a 4*S*-configuration.* Since the relative configuration of several lactones in this series is known,[16] the following configurations can be allocated: (3*R*,4*S*)-(−)-proto-lichesterinic acid,* (3*S*,4*S*)-(−)-alloprotolichesterinic acid,* and (2*R*,3*S*,4*S*)-nephromopsic acid.*

EXPERIMENTAL

Ranunculin. From 200 g of *Pulsatilla vulgaris* Mill. 1.6 g of ranunculin was isolated according to the method of Hill and van Heyningen.[1] M.p. 140−141°, $[\alpha]_D^{25}$ −81.0° (c=0.961, H_2O) (Lit.[1] m.p. 141−142°, $[\alpha]_D^{17.5}$ −80.7°). IR spectrum identical to that published by Bredenberg.[3] $UV_{max}(H_2O)$ 204 nm (log ε 4.00) (Lit.[3] UV_{max} 205 nm (log ε 3.99)).

(S)-*5-Hydroxy-2-penten-4-olide.* The hydrolysis was performed essentially according to Hellström.[4] To 100 mg of emulsin in 10 ml of phosphate buffer (pH=5.9) was added 200 mg of ranunculin. The reaction was followed polarimetrically. After 8 h a constant value of $[M]_D^{25}$ −65° was obtained. Assuming the presence of only mutarotated D-glucopyranose ($[M]_D$ +95° [17]) and the genuine aglucone, the $[M]_D^{25}$ of the aglucone is calculated to −160° (Lit.[4] −160°). The hydrolysate was extracted with 3×30 ml of ether, the pooled ether extracts dried over sodium sulfate and evaporated *in vacuo* at 25°. 63 mg of colourless oil was obtained. R_F=0.51 (Whatman No. 1 paper; 1-pentanol-85 % formic acid (1:1); detecting agent: ammoniacal silver nitrate. (Lit.[4] 0.55). $[\alpha]_D^{25}$ −145° (c=0.126, H_2O); $[M]_D^{25}$ −148°.

Periodate oxidation of ranunculin. a) Ranunculin (30 mg) was dissolved in 1 ml of water, and the solution was mixed with 1 ml of a solution containing 150 mg of sodium periodate. The reaction was followed polarimetrically and within 40 min the rotational value was constant at $[M]_D^{25}$ −314°. $UV_{max}(H_2O)$ 204 nm (log ε 4.11) indicating that the aglucone was not attacked.

b) The analytical determination of periodate oxidation was performed according to Guthrie [18] in an acetate buffer solution at pH 4.0. The unreduced periodate was determined according to Fleury-Lange. The consumption per mole of ranunculin was 2.3 moles of periodate. The determination of formaldehyde in the periodate oxidation mixture according to the acetylacetone-ammonia method [19] gave no result.

*Tetraacetyl-*L*-ascorbic acid.* L-Ascorbic acid was acetylated with acetic anhydride and perchloric acid according to Fritz and Schenk.[20] The NMR spectrum indicated

* The numbering used implies that the compounds are considered as derived from a butanolide and butenolide, respectively.

complete acetylation. UV_{max}(MeOH) 223 nm (log ε 3.54) and shoulder at 255 nm (log ε 3.11). $[\alpha]_D^{25}$ −46.0° (c=0.217, H_2O).

(S)-*Lichesterinic acid*. M.p. 104—106°, $[\alpha]_D^{25}$ −29.3° (c=0.209, $CHCl_3$), UV_{max}(MeOH) 227 nm (log ε 4.81) and shoulder at *ca.* 250—270 nm (log ε 3.54). Purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A.

Circular dichroism curves. Tetraacetyl-L-ascorbic acid, ranunculin, and (*S*)-γ-methyltetronic acid [UV_{max}(MeOH) 250 nm (log ε 4.19)] were measured on the Roussel-Jouan Dichrograph CD 185 and (*S*)-α-acetyl-γ-methyltetronic acid [UV_{max}(MeOH) 230 nm (log ε 4.17) and 265 nm (log ε 4.21)] and (*S*)-lichesterinic acid on the older model covering the 220—600 nm spectral range. The solvent was methanol and the concentration used was in the range of 2×10^{-3} M to 3×10^{-3} M except for (*S*)-α-acetyl-γ-methyltetronic acid, which was measured in a concentration of 7.0×10^{-5} M point by point and not automatically.

NMR spectra. The 60 Mc/s spectra were recorded on a Varian A 60 instrument. The solution of *ca.* 40 % deuterium bromide in deuterium oxide was prepared from 540 mg of phosphorus tribromide and 1 ml of deuterium oxide.

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REFERENCES

1. Hill, R. and van Heyningen, R. *Biochem. J.* **49** (1951) 332.
2. Kuhn, R. and Löw, I. *Ber.* **74** (1941) 219.
3. Bredenberg, J. B. *Suomen Kemistilehti* **B 34** (1961) 80.
4. Hellström, N. *Kgl. Lantbruks-Högsk. Ann.* **25** (1959) 171.
5. Benn, M. H. and Yelland, L. J. *Can. J. Chem.* **46** (1968) 728.
6. Jackson, E. L. and Hudson, C. S. *J. Am. Chem. Soc.* **59** (1937) 994.
7. McClenahan, W. S. and Hockett, R. C. *J. Am. Chem. Soc.* **60** (1938) 2061.
8. Reeves, R. E. *J. Am. Chem. Soc.* **71** (1949) 215.
9. Reeves, R. E. *J. Am. Chem. Soc.* **76** (1954) 4595.
10. Weiss, U. and Ziffer, H. *J. Org. Chem.* **28** (1963) 1248.
11. Bucourt, R., Legrand, M., Vignau, M., Tessier, J. and Delaroff, V. *Compt. Rend.* **257** (1963) 2679.
12. Arigoni, D., Daehne, W. v., Godtfredsen, W. O., Melera, A. and Vangedal, S. *Experientia* **20** (1964) 344.
13. Horii, Z., Tanaka, T., Tamura, Y., Saito, S., Matsumura, C. and Sigimoto, N. *Yakugaku Zasshi* **83** (1963) 602.
14. Boll, P. M., Sørensen, E. and Balieu, E. *Acta Chem. Scand.* **22** (1968) 3251.
15. Asahina, Y. and Azumi, T. *Ber.* **70** (1937) 1053.
16. Tamelen, E. E. v. and Bach, S. R. *J. Am. Chem. Soc.* **80** (1958) 3079.
17. Bates, F. J. and Associates. *Polarimetry, Saccharimetry and the Sugars*, United States Printing Office, New York 1942.
18. Guthrie, R. D. In Whistler, R. L. and Wolfrom, M. L. *Methods in Carbohydrate Research*, Academic, New York 1962, Vol. I, p. 435.
19. Speck, J. C. In Whistler, R. L. and Wolfrom, M. L. *Methods in Carbohydrate Research*, Academic, New York 1962, Vol. I, p. 441.
20. Fritz, J. S. and Schenk, G. H. *Anal. Chem.* **31** (1959) 1808.

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