

Spectroscopic Studies on Enols

4*. Hydrogen Bonding in Usnic Acid

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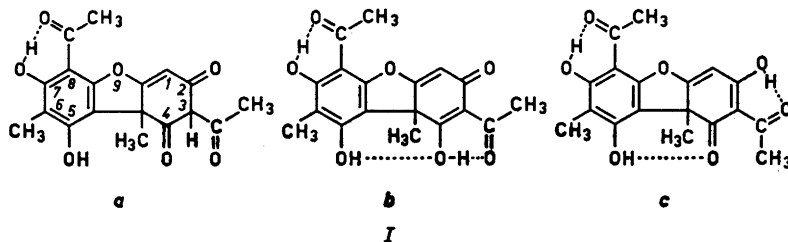
The acetylation of usnic acid has been reinvestigated. A new monoacetate is described and the positions of the *O*-acetyl groups in both acetates are established by spectroscopic methods.

Infrared and proton magnetic resonance spectra show that usnic acid contains three intramolecular hydrogen bonds. The strongest of these is found in the enolised β -triketo-part of the molecule. The hydrogen bond in the aromatic *o*-hydroxyacetyl grouping is stronger than in *o*-hydroxyacetophenone. The third hydrogen bond connects the remaining phenolic hydroxyl group with the tricarbonyl system. As shown by paper chromatography the lipophilicity of usnic acid is markedly enhanced when this hydroxyl group is acetylated.

Among β -triketones of natural origin, usnic acid (I) is of special interest due to its very wide occurrence — both enantiomers being represented — in lichens of many genera and due to its extreme toxicity. The now accepted structure was proposed independently by Curd and Robertson¹ and by Schöpf and Ross^{2,3} but has only recently been confirmed through a total synthesis of usnic acid by Barton, Deflorin and Edwards⁴. The extensive literature on the subject has been reviewed repeatedly, most recently by Shibata⁵ and by Hassall⁶.

Structure (I) (dibenzofuran numbering) obviously implies several possibilities for formation of intramolecular hydrogen bonds. In fact usnic acid, in spite of its high oxygen content, exhibits pronounced lipophilic properties. It is practically insoluble in the lower alcohols but somewhat soluble in benzene and readily soluble in chloroform.

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Usnic acid on mild treatment with acetic anhydride and sulphuric acid affords 5,7-*O,O*-diacetylusnic acid ⁷ which by treatment with sodium carbonate solution readily affords a monoacetate, 5-*O*-acetyl- or 7-*O*-acetylusnic acid, in excellent yield ⁷. So far, the position of the acetyl group has not been settled. However, since the phenolic groups in usnic acid would be expected to differ considerably in character, some information might be obtained directly from a consideration of the solubility properties. Such a study proved possible in a simple way by means of paper chromatography, using dimethyl sulphoxide as a stationary phase.

The pronounced trailing of usnic acid, indicative of the strong metal chelating properties of these compounds, was largely eliminated by the use of paper impregnated with ethylenediamine tetraacetic acid (EDTA), as previously tried with advantage in the chromatography of tropolones ⁸.

As demonstrated by the R_F -values obtained (Table 1), the elimination of one acetyl group from the diacetate causes a remarkable increase in the lipophilic character of the molecule. Hence, the liberated hydroxyl group is presumably largely masked by chelation, as expected in a 5-*O*-acetylusnic acid. Moreover, a reinvestigation of the acetylation reaction revealed the transient occurrence in the reaction mixture of a second monoacetate, presumably 7-*O*-acetylusnic acid, characterised by lower R_F -values even than usnic acid (Table 1). The (–)-form and the racemic form of this derivative were eventually obtained in a pure state.

The dominant role of intramolecular hydrogen bonding in the usnic acid series was still more obvious in view of the highly different properties of the two monoacetates. This suggested a closer investigation using spectroscopic methods. Surprisingly infrared spectroscopy has been little used even in recent work on usnic acid, although its IR spectrum has been published without discussion ⁹.

Previous investigations in this series of the hydrogen bonding in enolised tricarbonyl systems using infrared and proton magnetic resonance spectroscopy have recently been summarised ¹⁰ and provide a necessary background for the interpretation of the complicated spectra of usnic acid. A spectroscopic investigation of the acetates also led to the definite localisation of the acetyl groups in the two monoacetates and gave a more complete understanding of the hydrogen bonding in this unique system.

EXPERIMENTAL

Methods

The PMR spectra were recorded on a Varian V-4 300 spectrometer operating at 40 Mc/s and equipped with a Varian K 3 519 field homogeneity control unit. The measurements were made at $22 \pm 1^\circ\text{C}$. The shifts were determined against an internal tetramethylsilane standard and are given as τ -values according to Tiers¹¹.

The IR spectra were recorded on a Perkin Elmer No 21 instrument with a sodium chloride prism for 0.1 M solutions in chloroform using mostly 0.1 mm and 1 mm cells. The solvent absorption was compensated.

Paper chromatograms were run on paper (Whatman No. 4) impregnated with an EDTA buffer and dimethyl sulphoxide, using a technique previously described⁸. Hexane and di-isopropyl ether were used as mobile phases. The samples were applied as 1 % solutions in chloroform. The spots were located by means of a UV lamp after drying the paper at 80° . Usnic acid and its derivatives appeared as dark spots. The approximate R_F -values are listed in Table 1.

Table 1. Approximate R_F -values at 25° .

| | Mobile phase | |
|---|--------------|-------------------|
| | hexane | di-isopropylether |
| (\pm)-Usnic acid | 0.10 | 0.25 |
| (\pm)-5- <i>O</i> -acetylusnic acid | 0.55 | 0.85 |
| (\pm)-7- <i>O</i> -acetylusnic acid | 0.03 | 0.15 |
| (\pm)-5,7- <i>O,O</i> -diacetylusnic acid | 0.15 | 0.60 |

Materials

(\pm)-Usnic acid. (-)-Usnic acid, m.p. $203-204.5^\circ$, $[\alpha]_D^{20} = -498^\circ$ (chloroform, c , 0.4) from *Haematomma coccineum* (Dicks.) Körb. was racemised by prolonged heating in xylene and was crystallised from chloroform-ethanol to give prisms, m.p. $193-194^\circ\text{C}$.

(\pm)-5,7-*O,O*-diacetylusnic acid⁷. (\pm)Usnic acid (5.0 g) was stirred with acetic anhydride (100 ml) containing sulphuric acid (0.4 g) for 7 h at 20° . The crystals were collected, washed with cold methanol and water and dried (yield 3.5 g). Crystallisation from ethyl acetate gave (\pm)-5,7-*O,O*-diacetylusnic acid as yellowish needles, m.p. $205-207^\circ$ after sintering from 200° .

(\pm)-5-*O*-acetylusnic acid⁷. The diacetate (1.0 g) was stirred with 1 M sodium carbonate solution (150 ml) for 2 h at 25°C . The clear solution was acidified with hydrochloric acid, the precipitate collected, washed with water and dried (yield, 0.9 g). Crystallisation from ethyl acetate gave (\pm)-5-*O*-acetylusnic acid as yellow needles, m.p. $190-191^\circ$.

(\pm)-7-*O*-acetyl usnic acid. Finely pulverised (\pm)-usnic acid (2.3 g) was stirred at 20°C with acetic anhydride (60 ml) containing sulphuric acid (0.2 g). After 20 min, the solution was freed from undissolved usnic acid by rapid filtration through glass wool and poured with stirring into ice-water (1 200 ml). The precipitate was collected, washed with water and dried (yield 2.1 g). Crystallisations from methyl ethyl ketone and finally from ethyl acetate afforded (\pm)-7-*O*-acetylusnic acid as pale yellow needles, m.p. $182-183^\circ$ (yield 0.25 g). (Found: C 62.5; H 4.69. $\text{C}_{20}\text{H}_{18}\text{O}_8$ requires C 62.2; H 4.67.)

(-)-7-*O*-acetylusnic acid. Finely pulverised (-)-usnic acid (10 g) was stirred at 24°C with acetic anhydride (200 ml) containing sulphuric acid (0.8 g). After 15 min, undissolved usnic acid (4.0 g) was collected as above and the clear solution poured with stirring into ice-water (2 000 ml). The resinous product was dissolved in ethyl acetate (300 ml). The solution was washed with water (4×50 ml) and evaporated to a syrup which was dissolved in hot methanol. The solution on standing in the open air very slowly deposited crystals which were collected (m.p. $120-128^\circ$, yield 2.7 g). Crystallisations from methanol, ethyl acetate-methanol (1:4) and finally from ethyl acetate gave (-)-7-*O*-acetyl usnic acid as yellow prisms, m.p. $137-139^\circ$, $[\alpha]_D^{20} = -422^\circ$ (CHCl_3 , $c = 1.1$) (yield 0.45 g). (Found: C 62.3; H 4.66. $\text{C}_{20}\text{H}_{18}\text{O}_8$ requires C 62.2; H 4.67.)

RESULTS

IR and PMR spectra were recorded for usnic acid, its two monoacetates and the diacetate. For comparison, the PMR spectra of 2-hydroxyacetophenone, 2,4-dihydroxyacetophenone and 2,4-dimethoxy-6-hydroxyacetophenone were measured. Previous measurements in this series have mostly been made on carbon tetrachloride solutions, but due to limited solubilities of the compounds mentioned this solvent was only used in a few cases. The structures of the two monoacetates were fully established by these measurements as indicated below. The spectra also indicate the complete enolisation of the β -triketo grouping in the solvents used. IR spectra of the solid compounds, taken on potassium bromide discs, indicate that enolisation is complete also in the solids.

The IR spectra of usnic acid and its derivatives show a number of peaks in the carbonyl region (the figures refer to notations in the spectra, Figs. 1a–d).

(1) The conjugated chelated carbonyl of the enolised β -trikarbonyl grouping gives a broad band near $1\ 540\text{ cm}^{-1}$. Its position should be compared with those of ceroptene (5-methoxy-2-cinnamoyl-6,6-dimethyl-cyclohex-4-ene-1,3-dione) and its transformation products at $1\ 515\text{--}1\ 525\text{ cm}^{-1}$.¹²

(2) The enol ether double bond absorbs near $1\ 610\text{ cm}^{-1}$ as in dimedone methyl ether and the ceroptene derivatives. Absorption of the aromatic ring can also be expected in this region.

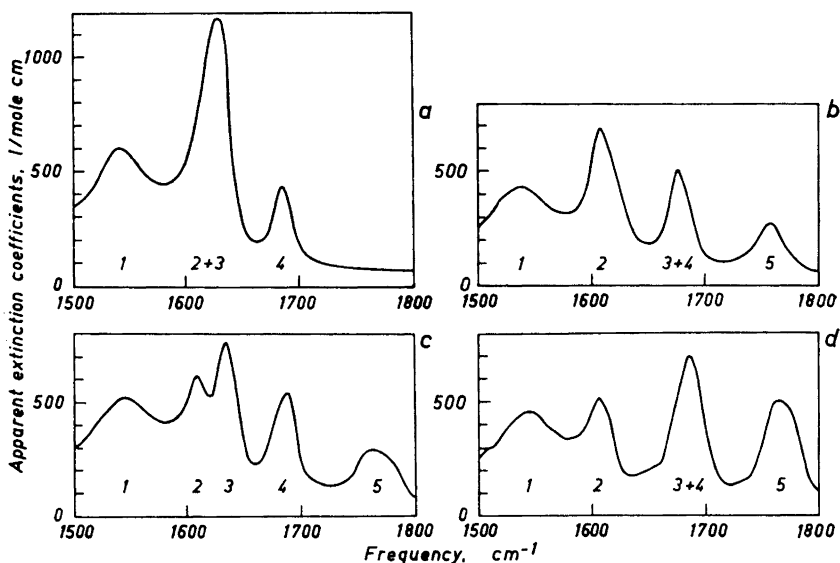


Fig. 1. Carbonyl region in the infrared spectra measured in chloroform solutions. (a) Usnic acid; (b) 7-O-acetylusnic acid; (c) 5-O-acetylusnic acid; (d) 5,7-O-O-diacetylusnic acid. The numbers 1–5 refer to discussion in the text.

(3) The aromatic *C*-acetyl group in 2,4-dimethoxy-6-hydroxyacetophenone absorbs near $1\,620\text{ cm}^{-1}$. In usnic acid the corresponding band coalesces with the enol ether band and a very intense absorption is observed. On acetylation of the *ortho*-hydroxy group the band is displaced to *ca.* $1\,680\text{ cm}^{-1}$, as expected for a non-chelated aromatic ketone. Acetylation of the *para*-hydroxyl group also affects the carbonyl absorption and in 5-*O*-acetylusnic acid all the bands can be readily seen.

(4) The dienone carbonyl absorbs near $1\,670\text{ cm}^{-1}$.

(5) The *O*-acetyl groups, as expected, absorb near $1\,780\text{ cm}^{-1}$.

The hydroxyl stretching absorptions of enolised β -tricarbonyl compounds are generally far less distinct than those of other chelates. The bands are broad, of low extinction and displaced towards unusually low frequencies. The $2\,000\text{--}4\,000\text{ cm}^{-1}$ region of the IR spectra of the present compounds (Fig. 2) shows three different hydroxyl absorptions, occurring at *ca.* $2\,400$, $2\,800$ and $3\,000\text{--}3\,200\text{ cm}^{-1}$. The first absorption can tentatively be assigned to the very strongly hydrogen bonded enolic hydroxyl group. The absorption in the $2\,800\text{ cm}^{-1}$ region agrees with that observed in 2,4-dimethoxy-6-hydroxyacetophenone and therefore can be ascribed to the chelated 7-hydroxy group. The third absorption is shown only by usnic acid and the 7-*O*-acetyl-derivative and must be attributed to the 5-hydroxy group. The position of the band indicates that this hydroxyl group is also engaged in an intramolecular hydrogen bond of by no means negligible strength.

The IR spectra can thus be satisfactorily interpreted and reveal that usnic acid contains three quite distinct hydrogen bonds of different character. Spectra recorded for different concentrations showed no significant changes and it is therefore evident that these hydrogen bonds are intramolecular.

The PMR spectra are very distinct. The figures refer to notations in the spectra of the chloroform solutions (Figs. 3*a*–*d*). The signals from the hydroxyl protons afford straight-forward information about the hydrogen bonding.

(1) The hydroxyl proton of the triketone chelate gives a signal at very low field — for usnic acid at $\tau = -9.20$. For the more dilute carbon tetrachloride solution this signal was observed at $\tau = -8.94$. The signals should be compared with those of ceroptene and its transformation products* and indicate that the enolic hydrogen bond in usnic acid is comparable to the hydrogen bonds in these compounds. The signal is shifted somewhat towards higher field on acetylation of the phenolic hydroxyl groups, especially that in the 5-position.

(2) The phenolic proton of the aromatic conjugated chelate gives a signal at surprisingly low field. Some related compounds were investigated for comparison; thus 2-hydroxyacetophenone, 2,4-dihydroxyacetophenone and 2,4-dimethoxy-6-hydroxyacetophenone in chloroform solutions gave signals for the chelated protons at $\tau = -2.32$, -2.70 and -4.03 . The latter values agree in magnitude with those observed in usnic acid and in 5-*O*-acetylusnic

* Ceroptene in carbon tetrachloride solution has been reported¹² to give an enol proton signal at $\tau = -8.91$. Its PMR spectrum has now also been recorded for a more concentrated chloroform solution. Enol proton signals were observed at $\tau = -9.31$ and -9.05 . The intensity ratio of 4/1 was also found for the other split peaks in the spectrum.

acid. The position of these signals imply that the hydrogen bonds in these aromatic chelates are rather strong. It should be remembered, however, that diamagnetic anisotropic effects of the aromatic system contribute to the low-field position.

(3) This signal is obviously due to the phenolic hydroxyl group in the 5-position. Its position at a fairly low field indicates that it is engaged in a hydrogen bond comparable in strength to that in salicylaldehyde. This is in agreement with the infrared hydroxyl absorption pattern.

Signal (4), which recurs in all spectra, is obviously due to the proton in the 1-position.

The signals from the methyl groups are generally well separated. Comparison with the model compounds, 2,4-dimethoxy-6-hydroxyacetophenone and 3-methyl-2,4,6-trihydroxyacetophenone, indicates that these signals in usnic acid are, from lower field, coinciding signals from the *C*-acetyl groups, the aromatic methyl and the angular methyl group.

CONCLUSIONS

The IR and PMR spectra thus further confirm the structure (I) for usnic acid and give unambiguous evidence concerning the position of the *O*-acetyl groups in the earlier known monoacetate, 5-*O*-acetylusnic acid, and in the new isomer, 7-*O*-acetylusnic acid. Moreover the demonstration of three intramolecular hydrogen bonds of quite different types also accounts for the pronounced lipophilicity of usnic acid. The exceptionally strong hydrogen bonding in enolised β -tricarbonyl systems has been repeatedly demonstrated in this series. The hydrogen bond in the aromatic chelate is even more pronounced than expected. Unfortunately, only limited information is available on hydrogen bonding in substituted *ortho*-hydroxyacetophenones and related compounds. Our results

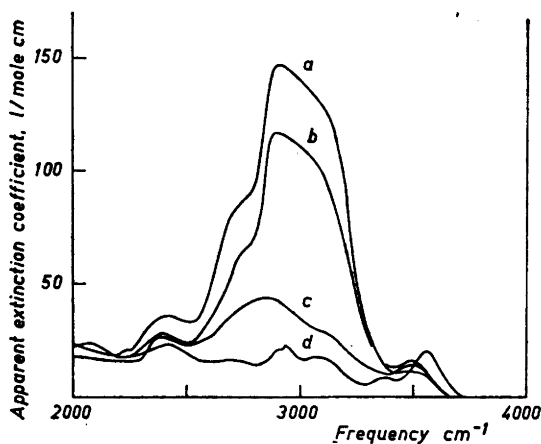


Fig. 2. Hydroxyl region in the infrared spectra of (a) usnic acid; (b) 7-*O*-acetylusnic acid; (c) 5-*O*-acetylusnic acid; (d) 5,7-*O*-diacetylusnic acid. The details of the C—H absorptions between 3 000 and 2 900 cm^{-1} are obscured by solvent absorption.

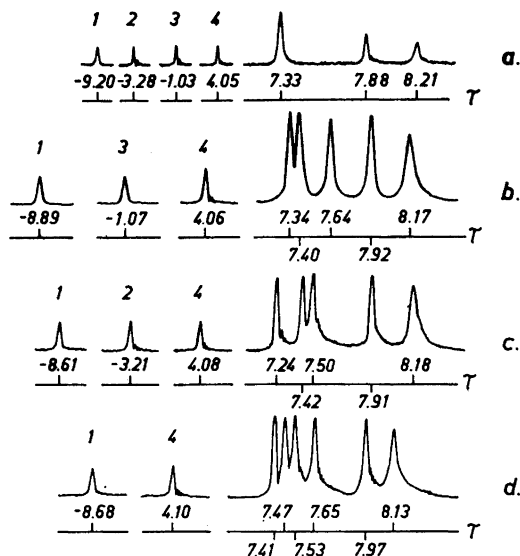


Fig. 3. PMR spectra recorded for chloroform solutions. (a) Usnic acid (mole fraction, $x = 0.02$); (b) 7-*O*-acetylusnic acid ($x = 0.04$); (c) 5-*O*-acetylusnic acid; (d) 5,7-*O*-diacetylusnic acid ($x = 0.04$).

and those obtained by Reeves *et al.*¹³ indicate that the strength of the intramolecular hydrogen bond in *o*-hydroxycarbonyl compounds is increased by hydroxy- or methoxysubstitution in *ortho*- or *para*-position to the carbonyl group and by nitro groups in *para*-position to the chelated hydroxyl group.

The third hydrogen bond in usnic acid is weaker but still strong enough to be comparable to that in salicylaldehyde. The reason for its strength is not directly apparent from the formulas drawn. Studies on molecular models, however, show that the distance from the oxygen in the 5-position to the oxygen in the 4-position is rather low. However, the 5-hydroxy-group is probably also capable of distinct intermolecular hydrogen bonding with strongly polar solvents. This is perhaps most obvious from the paper chromatographic data summarised in Table 1, where it is shown that the lipophilicity is strongly enhanced by acetylation of this group.

In an unsymmetrical β -triketone like usnic acid there are two possibilities for enolisation (I b and I c) (*cf.* Ref.^{10,12,14}). In some other β -triketones both forms have been observed to occur simultaneously by the splitting of the peaks in the PMR spectra. In usnic acid or its derivatives no such splitting is observed and it therefore seems probable that one of the forms strongly predominates in the solvents used. It is not possible, however, to judge from the present data whether usnic acid is present as form I b or I c.

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