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Methylation and Racemisation Studies on Usnic Acid

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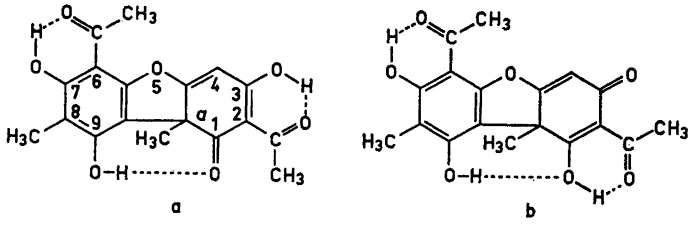
An improved method is presented for synthesis of (+)-8-*C*-methylusnic acid. In chloroform solution, this compound is present as a mixture of two tautomers.

Methylation of (+)-usnic acid with excess methyl iodide and potassium carbonate in acetone gives (+)-7-*O*-methylusnic acid, but 7,9-di-*O*-methylusnic acid could not be detected. The rates of racemisation of (+)-usnic acid, its 7-*O*-methyl, 8-*C*-methyl, 7-*O*-acetyl, 9-*O*-acetyl and 7,9-di-*O*-acetyl derivatives in dioxan indicate that the stability of usnic acid and its derivatives depends on the presence of an intramolecular hydrogen bond between the 9-hydroxyl group and the 1-oxygen atom.

Among the numerous compounds isolated from lichens, the optically active usnic acid (Ia or, less likely Ib) * is of interest as a racemisable compound containing a quaternary carbon atom where racemisation has been postulated to proceed through a ketene intermediate.[2] Usnic acid is also of biological interest since it is toxic in higher animals and has been found to exert hypermetabolic



I

activity on intravenous injection into anesthetised, immobilised cats and rabbits. This effect, its time course and the degree of respiratory augmentation is comparable to the well-known effect of 2,4-dinitrophenol.[3] Many enols with a pK_a around 5 belonging to a variety of types, *e.g.* vulpinic acid,[4]

* The numbering in formula Ia follows IUPAC Nomenclature of organic chemistry, section C (1965) (Rules 813. 3–4 and 824. 2–4), and differs from numbering in Ref. 1.

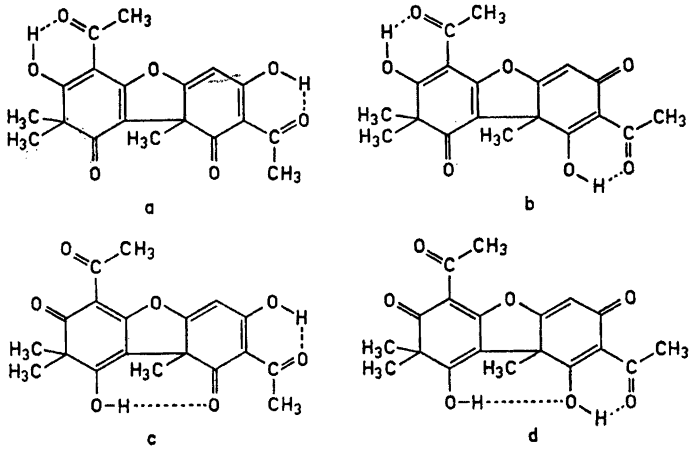
humulone,[5] dicoumarol,[6] 2-phenyl-indane-1,3-dione,[6] and butyrylfilicinic acid,[7] act *in vivo* in the same manner. The effect on the basal metabolic rate seems to be particularly pronounced in the case of strongly lipophilic enols.

2,4-Dinitrophenol and many of the compounds mentioned above act *in vitro* as so-called uncouplers of oxidative phosphorylation.[8,9] The relationship between the effects *in vitro* and *in vivo* is, however, still not clear. Usnic acid and its derivatives might be of value for a study of the relation between structure and physiological activity in this field.

Previous work[1] on usnic acid and on 7-*O*-acetyl-, 9-*O*-acetyl-, and 7,9-di-*O*-acetylusnic acids using IR and NMR spectroscopy has shown the presence in usnic acid of three intramolecular hydrogen bonds, two of which are in conjugated chelate rings. This hydrogen bonding accounts for the markedly lipophilic character of usnic acid. The phenolic hydroxyl groups in 7- and 9-positions show pK_a-values around 11 and 9, respectively[10] and are presumably not essential for the physiological activity. These groups should be available for substitution in seeking to obtain active derivatives with modified lipophilic character.

The present investigation deals mainly with attempts to prepare *O*-methyl derivatives of usnic acid which should be more stable towards hydrolysis *in vivo* than the acetates. However, while (+)-7-*O*-methylusnic acid could be prepared, it soon became clear that attempted *O*-methylation in the 9-position of (+)-7-*O*-methylusnic acid failed to give the expected product and resulted in decomposition reactions. The investigation was therefore extended to a study of underlying causes for this behaviour of the otherwise fairly stable ring system of usnic acid.

Dean *et al.*,[11] on methylation of (+)-usnic acid with dimethyl sulphate in aqueous sodium hydroxide solution, obtained a compound $C_{19}H_{18}O_7$, m.p. 136°, $[\alpha]_D^{20}$ +355° (chloroform), which they suggested was an *O*-methylusnic acid because of the results of the methoxyl group determination. Takahashi *et al.*[12] prepared the same compound but on the basis of degradative



II

Table 1. Proton chemical shifts (δ ppm) of (+)-usnic acid and methyl derivatives in $CDCl_3$ solutions. All signals are singlets.

Protons	(+)-Usnic acid [1] Ia or Ib	(+)-8-*C*-Methylusnic acid			(+)-7-*O*-Methylusnic acid
		without Et_3N		with Et_3N	
		IIa or IIb	IIc or IId		
8-$C(CH_3)_2$	—	1.45; 1.49	1.45; 1.49	1.45; 1.49	—
Angular CH_3	1.79	1.85	1.79	1.83	1.80
8-CH_3	2.12	—	—	—	2.20
2-$COCH_3$ / 6-$COCH_3$	2.67	2.53; 2.62	2.49; 2.67	2.58	2.64; 2.68
OCH_3	—	—	—	—	3.83
4-H	5.95	5.91	6.07	5.96	5.99
9-OH	11.03	—	12.30	—	10.90
7-OH	13.28	17.70	—	—	—
3-OH(1-OH?)	19.20	17.90	18.83	—	18.97

and spectral investigations reformulated it as the (+)-8-*C*-methylusnic acid, form IIa.

We have synthesised this compound, using essentially the method of Dean *et al.*[11] However, as it was fairly unstable the isolation procedure was modified. If (+)-8-*C*-methylusnic acid exists in a single form, *e.g.* IIa, it would be expected to give only 7 or 8 signals in the NMR spectrum.* However, the spectrum (Table 1) contains 14 signals, indicating the occurrence in the $CDCl_3$ solution of two external tautomers.[14] This is confirmed by the addition of a small amount of triethylamine,[15] giving a simplified spectrum which contains average signals of the tautomers.

There is no signal from methoxyl protons in the spectra and no signal from aromatic methyl protons (at δ 2.12 ppm in usnic acid [1] (Table 1)) was observed. Instead, two close signals due to two geminally bound methyl groups are found, in agreement with the structure proposed.

The four low-field signals at δ 12.30 (0.25 H), 17.70 (0.75 H), 17.90 (0.75 H), and 18.83 ppm (0.25 H) from the compound in pure $CDCl_3$ solution, which would be due to hydrogen-bonded hydroxyl protons, indicate that both tautomers are completely enolised. The tautomers can be represented by two of the formulas IIa—d. The signals of the enol protons in the β-triketosystem of the two tautomers ought to be at very low field, as in usnic acid (δ 19.20 ppm) and its acetates (δ 18.61—18.89 ppm).[1] Corresponding signals from the major and minor tautomers should be those at δ 17.90 and 18.83 ppm, respectively. The signal at δ 12.30 ppm from the minor tautomer must be due to the enol proton in the 9-position of structures IIc or IId. Formula IId, however, implies a hydrogen bond between two enolic hydroxyls and appears less probable than IIc. The major tautomer should be ascribed to one of the remaining

*Takahashi *et al.*[13] report a single signal in the spectrum (δ 5.92 ppm, referred to the ethylenic proton).

structures IIa or IIb. The two tautomers are present in the $CDCl_3$ solution in the proportions 3:1 from the relative intensities of corresponding signals.

Investigations by, *e.g.*, Kornblum *et al.*[16,17] have shown that the nature of the solvent is an important factor in determining the course of reaction of ambident phenol anions with alkylating agents. Polar aprotic solvents favour *O*-alkylation while hydroxylic solvents cause increased *C*-alkylation, probably by solvating the phenolate oxygen atoms through hydrogen bonding.

(+)-Usnic acid in boiling acetone reacted slowly with a large excess of methyl iodide and potassium carbonate, giving a complex reaction mixture. After a reaction time of 60 h, when almost all usnic acid had reacted, the reaction product in chloroform was extracted first with sodium hydrogen carbonate and then with sodium hydroxide solutions.

The oily fraction isolated from the sodium hydroxide extract was chromatographed on a polyamide column to give a main fraction (20 %) of (+)-7-*O*-methylusnic acid, m.p. 92.5—93.5°. Apart from the methoxyl values (see experimental part), this gave analytical figures in accordance with a formula $C_{18}H_{15}O_6 \cdot OCH_3$ and its mass spectrum showed the expected molecular ion, $M^+ = 358$ m.u. All signals in the NMR spectrum (Table 1) except that at δ 3.83 ppm (3 H) due to methoxyl protons, have almost exact counterparts in the spectrum of usnic acid. As there is no signal around δ 13 ppm from a hydroxyl proton in 7-position, the compound is (+)-7-*O*-methylusnic acid.

From the acidified sodium hydrogen carbonate extract, a white, markedly polar compound, m.p. 162—167°, $[\alpha]_D^{22}$ −185° (methanol), was isolated in low yield. Its mass spectrum gave a molecular ion $M^+ = 376$ m.u., indicating the addition to usnic acid of the elements of methanol. Since its NMR spectrum (CD_3OD solution) lacked a signal from an ethylenic proton in the region δ 5—7 ppm, its formation probably involves addition of methanol or water to the ethylenic linkage of usnic acid. It was not investigated further.

The neutral chloroform solution yielded a syrup of low optical rotation. TLC of the syrup gave at least 6 spots which all gave yellow fluorescence under UV-light.

No spot due to 7,9-di-*O*-methylusnic acid was observed in this experiment. This dimethyl ether would be expected to exhibit similar properties to those of 7-*O*-acetyl-, 9-*O*-acetyl-, 7,9-di-*O*-acetyl-, and 7-*O*-methylusnic acids. These, like usnic acid itself, are readily extracted from a chloroform solution with aqueous sodium hydroxide and give dark spots under UV-light on thin-layer and paper chromatography.

Attempts were also made to synthesise the dimethyl ether of usnic acid by further treatment of (+)-7-*O*-methylusnic acid with methyl iodide and potassium carbonate in boiling acetone for 60 h. The alkali-soluble part of the reaction mixture contained only 7-*O*-methylusnic acid. The alkali-insoluble fraction gave several spots on TLC, all of which gave yellow fluorescence under UV-light. (+)-7-*O*-Methylusnic acid, when treated similarly with potassium carbonate in boiling acetone, gave no decomposition products. Similar results were obtained, when dimethyl sulphoxide was used as solvent to further promote *O*-alkylation.

The decomposition observed in these experiments might be due to *C*-methylation in the aromatic ring with the formation of products as unstable

Table 2. Racemisation of (+)-usnic acid and some derivatives in dioxan.

Expt.	Compound	Temp. in °C	Conc. in mM	α_0	$10^2 \times k$, h^{-1}	Time in h, final	α/α_0 in %, final
1	(+)-Usnic acid	79.5	7.15	1.089	0.55	10	94.6
2	(+)-7-*O*-Acetylusnic acid	79.5	7.62	1.165	0.24	10	97.7
3[a]	(+)-9-*O*-Acetylusnic acid	60.5	7.00	0.706	11.3	6	35.9
4[a]	—»—	79.5	3.73	0.356	107	2	11.2
5	(+)-7,9-Di-*O*-acetylusnic acid	60.5	4.12	0.396	5.90	5	74.5
6	—»—	79.5	4.03	0.337	60.3	3	16.3
7[a]	(+)-8-*C*-Methylusnic acid	60.5	4.86	0.304	158	2	4.0
8	(+)-7-*O*-Methylusnic acid	79.5	5.42	0.449	0.30	12	96.5

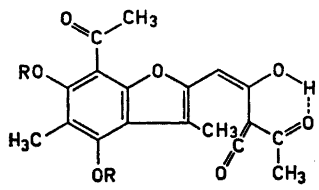
[a] The racemisation is accompanied by a slow decomposition as established by TLC.

as 8-*C*-methylusnic acid. However, the reaction conditions used would favour *O*-alkylation and it seems reasonable to assume that the unknown 7,9-di-*O*-methylusnic acid also would be decomposed as fast as it is formed.

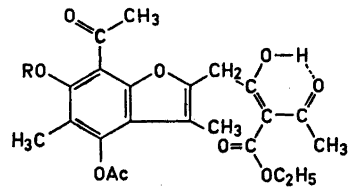
Usnic acid has for a long time been known to undergo several reactions where cleavage of bond *a* (Ia) occurs with formation of benzofuran derivatives.[18] Stork[2] has given an interpretation for these reactions involving splitting of bond *a* with formation of a ketene intermediate (III) and has also postulated that the racemisation of usnic acid could be accounted for by the same intermediate. An estimation of the relative stabilities of (+)-usnic acid and its derivatives might thus be obtained by a comparison of their rates of racemisation.

MacKenzie[19] observed that the (+)-diacetate was racemised more rapidly than (+)-usnic acid in dioxan. The present study of the racemisation of (+)-usnic acid and some derivatives (Table 2) was carried out on dioxan solutions using a photoelectric polarimeter. The observed first order constants (k) of the racemisation reactions (compare note in Table 2) were obtained graphically from the straight lines $\ln \alpha_0/\alpha = kt$.

Acetylation of the hydroxyl group in position 7 of (+)-usnic acid (Expts. 1 and 2) and of (+)-9-*O*-acetylusnic acid (Expts. 3 and 5; 4 and 6), respectively, gives about 50 % decrease in k. Methylation of the 7-hydroxyl group in (+)-usnic acid (Expts. 1 and 8) also results in a small decrease in k. In these three



III



IV R=H
V R=Ac

cases the carbonyl group at position 6 has been changed from strongly chelated to unchelated resulting in a somewhat increased electron-withdrawing capacity.

An opposite and greater effect is caused by acetylation of the hydroxyl group in position 9 of (+)-usnic acid (Expts. 1 and 4) and (+)-7-*O*-acetylusnic acid (Expts. 2 and 6). The observed increase in k by factors of 195 and 250, respectively, can be explained by a disappearance of the hydrogen bond between the 9-hydroxyl group and the 1-oxygen atom of the β-tricarbonyl system. This bond, although not of the conjugated chelate type, is somewhat unexpectedly comparable in strength to the hydrogen bond in salicylaldehyde.[1] Racemisation of usnic acid and its derivatives with a free 9-hydroxyl group *via* a ketene intermediate (III) would require a rupture of this hydrogen bond already in the transition state.

MacKenzie [19] observed that the racemisation of (+)-usnic acid increased in rate with the basicity of the solvent for eight different solvents and gave corresponding values of activation energies and entropies. These results can be interpreted in a similar way. In a decaline or toluene solution, the 9-hydroxyl group of an activated complex would be essentially free. However, in, *e.g.*, a dioxan solution, this hydroxyl group should be hydrogen bonded to a solvent molecule, causing a decreased activation energy for the racemisation reaction in a solvent of this type.

The reactivities of (+)-usnic acid and some derivatives have been compared in boiling ethanol where the (+)-9-*O*-acetyl [20] and (+)-7,9-di-*O*-acetyl [21] derivatives were early reported to give high yields of ethanol addition products by boiling for 4—5 h. The present results have confirmed the high reactivities of these two rapidly racemised acetates. The NMR spectra of the reaction products support structures (IV) and (V) (*cf.* Asahina and Shibata [22]). (+)-Usnic acid, (+)-7-*O*-acetyl-, and (+)-7-*O*-methylusnic acids, however, could be recovered unchanged with practically no racemisation after boiling in ethanol for 10 h. These findings further support the same ketene type intermediate (III) in the racemisation and ethanol addition reactions.

Derivatives of usnic acid methylated in the 9-position would show the same easy rupture of bond *a* (Ia) as the corresponding acetates. The large amounts of neutral by-products of low optical rotation formed in the synthesis of (+)-7-*O*-methylusnic acid are thus not unexpected.

It is noteworthy that (+)-8-*C*-methylusnic acid which lacks the 9-hydroxyl group in one of the tautomers (IIa or IIb) is exceptionally easily racemised (Table 2, Expt. 7). Bond *a* in (+)-8-*C*-methylusnic acid is also reported to undergo facile cleavage in boiling methanol.[13]

The easy thermal racemisation of (+)-7,9-di-*O*-acetylusnic acid explains the formation of a racemate on acid-catalysed acetylation of (+)-usnic acid at 90° for 30 min.[21] Isolated observations of such acetylation experiments have been taken to indicate that racemisation of usnic acid is acid catalysed [23] but this seems to require further confirmation. Similarly, the m.p. 202° for (+)-7,9-di-*O*-acetylusnic acid [20] obviously refers to the racemate (m.p. 205—207°).[21] Cautious melting point determination in a preheated apparatus gives m.p. 156° for the (+)-7,9-di-*O*-acetyl derivative.

The present investigation was undertaken to study the possibilities of modifying the lipophilic character of usnic acid by *O*-methylation. Table 3

demonstrates that 7-*O*-methylusnic acid, unlike the 7-*O*-acetyl derivative, is somewhat more lipophile than usnic acid. On the other hand acetylation of the 9-hydroxyl group causes a marked increase in the lipophilic character of the products. However, the low stability of usnic acid derivatives lacking a free 9-hydroxyl group probably prevents their use in physiological experiments.

At an early stage in this work diazomethane was tried as a reagent to effect *O*-methylation of usnic acid. It was found to be unsuitable but an unexpected reaction product, $C_{19}H_{16}O_6$, m.p. 189—190.5°, containing a condensed furan ring, was isolated in 15 % yield.[24]

EXPERIMENTAL

Melting points were determined on a Kofler micro hot stage. When preheating was needed a Gallenkampf melting point apparatus was used. IR spectra were recorded on a Perkin-Elmer No. 221 and UV spectra in 99.5 % ethanol (0.025 mM solutions) on a Beckman DK 2 spectrophotometer. NMR spectra were measured on a Varian A-60 spectrometer operating at 60 Mc/s using $CDCl_3$ as solvent unless otherwise stated (10—12 % solutions). The sample temperature was 34±2°. Tetramethylsilane was used as internal standard and the resonance fields are reported as δ_{TMS}. Mass spectra were obtained on an LKB 9000 instrument with an all-glass heated inlet system at 20—80°.

All reactions were followed by thin-layer chromatography (TLC) on polyamide (Merck) with methanol as solvent. In some cases paper chromatography was used with dimethyl sulphoxide as stationary phase and hexane or di-isopropyl ether as mobile phase. The paper strips (Whatman No. 4) were impregnated with EDTA to diminish trailing.[1] The spots were detected by examining the plates or strips under UV-light (λ=254 nm) and then by spraying with 5 % iron(III) chloride in ethanol.

(+)-*Usnic acid.* (+)-Usnic acid (Schuchardt) was crystallised from chloroform-ethanol (1:1), m.p. 199—200°, $[\alpha]_D^{22}$ +497° (*c* 0.36, chloroform), mass spectrum (the largest peaks as percent of the base peak): 344 (M^+, 50 %), 260 (64 %), 233 (100 %), 217 m.u. (19 %).

(+)-8-C-*Methylusnic acid.*[11,12] To a solution of (+)-usnic acid (4.0 g) in aqueous sodium hydroxide (12.8 g in 135 ml water), dimethyl sulphate (24 ml) was added over a period of 1 h under vigorous stirring at 10°. After a further hour, the solution was acidified and the precipitate collected (3.95 g). The product was dissolved in chloroform (30 ml). The solution was extracted with 8 % sodium hydrogen carbonate solution (4×15 ml) and washed with 0.5 M hydrochloric acid and with water. The chloroform solution was dried and evaporated. The residue was crystallised from acetonitrile giving (+)-usnic acid (0.89 g), m.p. 198—200°, $[\alpha]_D^{21}$ +496° (*c* 0.40, chloroform).

The combined hydrogen carbonate solutions were acidified. The precipitate was collected to give chromatographically pure (+)-8-*C*-methylusnic acid (2.70 g). This was recrystallised twice from acetone at −30° and afforded (+)-8-*C*-methylusnic acid (0.61 g), m.p. 135—136° (decomp.; preheated to 125°, 3°/min), $[\alpha]_D^{22}$ +370° (*c* 0.23, chloroform), λ_{max}^{EtOH} 254 nm (ε 23 300), 305 nm (ε 15 600), $\lambda_{shoulder}^{EtOH}$ 358 nm (ε 10 400), ν_{max}^{KBr} 1690, 1650, 1600 cm^{-1}. Dean *et al.*[11] report m.p. 136°, $[\alpha]_D^{20}$ +355° (*c* 2.0, chloroform). Takahashi *et al.*[12] report m.p. 136°, $[\alpha]_D$ +364° (*c* 0.43, chloroform), λ_{max}^{EtOH} 258 nm (ε 22 000), 305 nm (ε 14 500), 358 nm (ε 12 400), ν_{max}^{KBr} 1690, 1650, 1603 cm^{-1}.

(+)-7-O-*Methylusnic acid.* Methyl iodide (57 g) was added with stirring to (+)-usnic acid (8.0 g) and dried potassium carbonate (30 g) in dry acetone (900 ml). The mixture was refluxed (60 h) until almost all usnic acid had reacted and was then poured into ice-water. The water solution was separated from excess methyl iodide, acidified and extracted with chloroform. The chloroform solution was extracted with 8 % sodium hydrogen carbonate (3×75 ml) (see below) and then with 1 M sodium hydroxide (4×100 ml).

The sodium hydroxide solutions were acidified and extracted with chloroform. The chloroform extract was washed with 1 M hydrochloric acid and water, dried and evaporated, yielding a syrup (3.30 g). This was fractionated on a polyamide column (Ø 50 mm; 190 g polyamide (Woelm) washed with methanol) using methanol as eluent. 50 ml fractions were collected and analysed by TLC. Fractions No. 23–37 were combined and evaporated to dryness and the residue was dissolved in chloroform. To remove low-molecular impurities from the polyamide, the solution was filtered through a short polyamide column. Evaporation of the filtrate gave a syrup (2.42 g) which was dissolved in methanol (25 ml). On the addition of water (4 ml) the solution slowly deposited crystals (1.5 g, m.p. 90–92°). Crystallisation from methanol-water (6:1) afforded (+)-7-*O*-methylusnic acid as pale yellow plates (0.90 g), m.p. 92.5–93.5°, $[\alpha]_D^{22}$ +460° (*c* 0.48, chloroform). (Found: C 63.8; H 5.00; OCH_3 7.13. $C_{18}H_{15}O_6 \cdot OCH_3$ (358.35) requires C 63.7; H 5.06; OCH_3 8.66). λ_{max}^{EtOH} 228 nm (ε 26 300), 268 nm (ε 26 000), $\nu_{max}^{CHCl_3}$ 1675, 1600, 1535 cm^{-1}, mass spectrum (the largest peaks as percent of the base peak): 358 (M^+, 72 %), 274 (100 %), 259 (25 %), 247 m.u. (99 %).

The methyl ether gives low methoxyl values by the Zeisel method, possibly because the methyl iodide formed reacts with the reactive aromatic ring of usnic acid even under acidic conditions.[25] It is interesting to note that Dean *et al.*[11] and Takahashi *et al.*[12] report high methoxyl values for (+)-8-*C*-methylusnic acid, 1.0 respective 0.6 methoxyl per mole. Similar results on *C*-methylated phloroglucinol derivatives are reported by Nilsson [26] and by Riedl and Risse.[25] It appears that nuclear methylation of phloroglucinol rings with methyl iodide under certain conditions may be a reversible reaction.

Unidentified by-products from the synthesis of (+)-*7*-O-*methylusnic acid.* The hydrogen carbonate solutions obtained as described above on the isolation of 7-*O*-methylusnic acid gave a crystalline fraction (250 mg). It was crystallised from 60 % aqueous methanol to give colourless needles (63 mg), m.p. 162–167°, $[\alpha]_D^{22}$ −185° (*c* 0.54, methanol) which were sparingly soluble in chloroform. Mass spectrum shows $M^+ = 376$ m.u.

The neutral chloroform solution from the same synthesis afforded a dark brown syrup (4.16 g), $[\alpha]_D^{22}$ +29° (*c* 0.6, chloroform). TLC of this showed at least 6 spots which gave yellow fluorescence under UV-light.

*Attempted synthesis of 7,9-di-*O-*methylusnic acid from* (+)-*7*-O-*methylusnic acid.* A mixture of (+)-7-*O*-methylusnic acid (35 mg), anhydrous potassium carbonate (120 mg), and methyl iodide (0.23 g) in dry acetone (8 ml) was refluxed for 60 h. The reaction mixture was poured into ice-water, acidified and extracted with chloroform. The chloroform solution was extracted with 1 M sodium hydroxide (3×5 ml). TLC on the chloroform phase gave a few spots which had yellow fluorescence under UV-light but no dark spot could be detected. The combined sodium hydroxide solutions were acidified and extracted with chloroform. Thin-layer and paper chromatography gave only one spot due to 7-*O*-methylusnic acid (Table 3).

A reaction mixture as above, but without methyl iodide was refluxed for 60 h, poured into ice-water, acidified, extracted with chloroform and dried. The residue contained only 7-*O*-methylusnic acid.

Experiments as above with dimethyl sulphoxide as solvent at 60° for 60 h gave the same results.

Table 3. Approximate R_F-values at 20° on thin-layer and paper chromatography.

Compound	TLC	Paper chromatography Mobile phase	
		hexane	di-isopropyl ether
(+)-Usnic acid	0.24	0.07	0.14
(+)-7-*O*-Acetylusnic acid	0.58	0.02	0.10
(+)-9-*O*-Acetylusnic acid	0.62	0.42	0.63
(+)-7,9-Di-*O*-acetylusnic acid	0.79	0.15	0.41
(+)-8-*C*-Methylusnic acid	0.20	0.01	0.05
(+)-7-*O*-Methylusnic acid	0.53	0.09	0.19

(+)-*7,9-Di-O-acetylusnic acid.* The diacetate of (+)-usnic acid was prepared according to Asahina and Yanagita,[20] keeping the temperature under 30°. The product was crystallised from methanol at −30° and gave (+)-7,9-di-*O*-acetylusnic acid, m.p. 156°, $[\alpha]_D^{22}$ +242° (*c* 0.52, chloroform), λ_{max}^{EtOH} 222 nm (ε 25 200), 256 nm (ε 19 000), $\lambda_{shoulder}^{EtOH}$ 308 nm (ε 9400). Asahina and Yanagita [20] report m.p. 202°, $[\alpha]_D^{30}$ +200° (*c* 0.94, chloroform). Barton and Bruun [23] report m.p. 203—204°, $[\alpha]_D$ +240° (*c* 2.85, chloroform), λ_{max}^{EtOH} 224 nm (ε 26 000), $\lambda_{infl.}^{EtOH}$ 249 nm (ε 17 000), 305 nm (ε 9000).

The (+)-diacetate melted at 203—205° on continuous heating, but on preheating to 156° or higher it melted at once and resolidified with remelting at 203—205°. Heating at 175° for 30 sec gave the racemate. The melting point of (±)-7,9-di-*O*-acetylusnic acid is reported as 205—207°.[21]

(+)-*9-O-Acetylusnic acid.* (+)-9-*O*-Acetylusnic acid was prepared according to Asahina and Yanagita [20] by partial hydrolysis of the (+)-diacetate in 1 M sodium carbonate solution, keeping the temperature under 25°. The product was crystallised from methanol at −30° and gave (+)-9-*O*-acetylusnic acid, m.p. 180—182°, $[\alpha]_D^{22}$ +286° (*c* 0.45, chloroform) λ_{max}^{EtOH} 231 nm (ε 27 900), 269 nm (ε 21 600), 351 nm (ε 7600). Lit.[20] m.p. 180—181°, $[\alpha]_D^{29}$ +292° (*c* 1.26, chloroform).

(+)-*7-O-Acetylusnic acid.* Pulverised (+)-usnic acid (4.0 g) was stirred at 22° with acetic anhydride (80 ml) containing sulphuric acid (0.3 g). After 15 min, the reaction mixture was freed from usnic acid (0.91 g) by filtration through glass wool and poured into ice-water. The resinous product was dissolved in ethyl acetate (120 ml). The solution was washed with water, dried and evaporated. The syrup was dissolved in hot methanol (40 ml). After 24 h at 20° the precipitate was collected (0.59 g) and was shown on TLC to be a mixture of usnic acid and diacetylusnic acid. The mother liquor was evaporated to 25 ml volume and on standing at 22° for 24 h it deposited crystals which were collected (m.p. 127—134°, yield 1.0 g). Crystallisations from methanol afforded (+)-7-*O*-acetylusnic acid (0.36 g) as pale yellow prisms, m.p. 137—139°, $[\alpha]_D^{19}$ +417° (*c* 0.41, chloroform), λ_{max}^{EtOH} 223 nm (ε 34 700), 267 nm (ε 30 100). Forsén *et al.*[1] report for the (−)-form: m.p. 137—139°, $[\alpha]_D^{20}$ −422° (*c* 1.1, chloroform).

Racemisation experiments. The racemisations of (+)-usnic acid and its derivatives were measured in purified dioxan [27] solutions (3—8 mM). The determinations were carried out in a jacketed polarimeter tube (10 cm) maintained at 60.5° or 79.5° (temperature in the tube) by circulating water from a thermostat. All measurements at 60.5° were run in sequence, before readjusting the thermostat to 79.5°. The optical rotations were determined with a Perkin-Elmer Model 141 photoelectrical polarimeter with light from a Hg-lamp (λ=578 nm). The final solutions were investigated by TLC. Ln α_0/α was plotted against time to give straight lines from which curves the first order constants (k) were determined graphically.

Treatment of (+)-usnic acid and derivatives with boiling ethanol. (+)-9-*O*-Acetylusnic acid (250 mg) in ethanol (7.5 ml) was refluxed for 4 h.[20] After cooling, the solution deposited yellow needles (210 mg). Crystallisation from ethanol gave "9-*O*-acetylusnic acid ethoxide" (IV) (178 mg), m.p. 112—113°, $[\alpha]_D^{21}$ 0° (*c* 0.28, chloroform) (lit.[20] m.p. 110°). NMR spectrum: δ 1.32 (3 H; triplet, J=7.0 cps), 2.07 (3 H), 2.17 (3 H), 2.38 (6 H), 2.78 (3 H), 4.22 (2 H), 4.28 (2 H; quartet, J=7.0 cps), 13.37 (1 H), 17.57 ppm (1 H).

(+)-7,9-Di-*O*-acetylusnic acid (250 mg) in ethanol (7.5 ml) was refluxed for 5 h.[21] After cooling, the solution deposited white needles (192 mg). Crystallisation from ethanol gave "7,9-di-*O*-acetylusnic acid ethoxide" (V) (168 mg), m.p. 90—93°, $[\alpha]_D^{21}$ 0° (*c* 0.35, chloroform) (lit.[21] m.p. 88—89°). NMR spectrum: δ 1.30 (3 H; triplet, J=7.0 cps), 2.03 (3 H), 2.19 (3 H), 2.33 (3 H), 2.38 (6 H), 2.68 (3 H), 4.25 (2 H), 4.26 (2 H; quartet, J=7.0 cps), 17.53 ppm (1 H).

(+)-Usnic acid (250 mg) after refluxing in ethanol (25 ml) for 10 h was recovered unchanged (230 mg) (m.p., IR ($CHCl_3$), specific rotation).

(+)-7-*O*-Acetylusnic acid (60 mg) after refluxing in ethanol (2 ml) for 10 h was recovered almost unchanged (52 mg), m.p. 132—137°, $[\alpha]_D^{21}$ +407° (*c* 0.25, chloroform). IR spectrum ($CHCl_3$) was identical with that of (+)-7-*O*-acetylusnic acid.

(+)-7-*O*-Methylusnic acid (60 mg) in ethanol (2 ml) was refluxed for 10 h. After adding water (0.3 ml) and cooling, the solution deposited starting material (48 mg), m.p. 91—

93°, $[\alpha]_D^{21}$ +458° (*c* 0.29, chloroform). IR spectrum ($CHCl_3$) was identical with that of (+)-7-*O*-methylusnic acid.

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