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COMPONENTS OF *SESELI SIBIRICUM*

CONSTITUTION AND SYNTHESIS OF SIBIRICIN, A NEW COUMARIN

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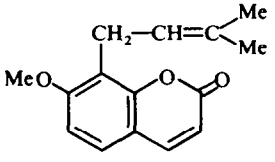
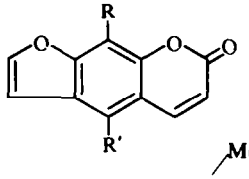
Abstract—From the pet. ether extract of *S. sibiricum* osthol, imperatorin, bergapten and a new coumarin named sibiricin have been isolated. It has been assigned 5,7-dimethoxy-coumarin-8-γ,γ-dimethylallyl epoxide structure on the basis of degradative and spectral studies. Final confirmation of the structure has been provided by a synthesis following an unambigous route using 3-dimethylallyl-2-hydroxy-4,6-dimethoxybenzaldehyde as the important intermediate.

Seseli sibiricum. Benth. (Umbelliferae) occurs mainly in Kashmir and nearby temperate localities. It is used for blending beverage and as a medicine for livestock.[1] The analysis of the essential oil of its rhizomes and roots was made by Handa *et al.*,[2] who reported the presence of fenchone, fenchyl alcohol and its acetate. The components of the umbels have now been studied. The pet. ether extract yielded four crystalline compounds after elaborate column chromatography supplemented by TLC. Out of these, three have been identified as osthol (I), imperatorin (II) and bergapten (III) by a study of their properties and comparison with authentic samples; the occurrence of these is very common in Umbelliferae.[3] The fourth crystalline compound which is new and optically active, m.p. 152–153°, has been named "Sibiricin". The subsequent benzene extract gave only one crystallizable compound, which was identified as osthol, while the alcohol extract yielded D-mannitol.



I



II: R = OCH_2—C=C(Me)Me; R′ = H

III: R = H; R′ = Me

Sibiricin has the mol. formula $C_{16}H_{18}O_5$ based on analysis and mol. wt. determination. It does not form an acetyl and DNP derivatives showing the absence of free hydroxylic and aldehydic or ketonic carbonyl groupings. Methoxyl estimation

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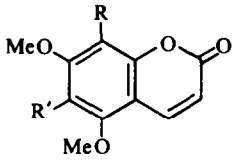
indicated two OMe groups. Comparison of its UV spectrum with those of known coumarins provided strong evidence for the presence of a 5,7-dioxygenated coumarin chromophore,[4,5] an assignment also supported by chemical reactions. It has IR absorption bands at 1740 and 1613 (unsaturated-δ-lactone), 1250 (epoxide) and 846 cm^{-1} (tetrasubstituted benzene).

Based on the above data sibiricin appears to have a 5,7-dimethoxycoumarin skeleton with a substituent in the 8 or 6-position and with epoxide in the side chain (IV or V). Confirmation of the epoxide was provided by the conversion of sibiricin into its corresponding glycol (VI–VII) when treated with 1% aq. oxalic acid. Structures V and VII were earlier assigned to aculeatin[6] and toddalolactone[7] respectively; sibiricin and its glycol are markedly different from these compounds and hence the remaining structures IV and VI respectively should be assigned to them.



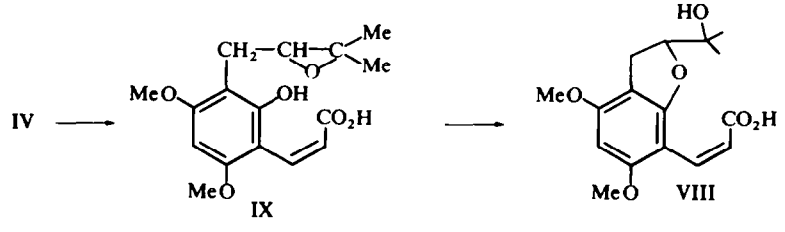
IV: R = —CH_2—CH(O)—C(Me)(Me); R′ = H

VI; R = —CH_2—CHOH—C(OH)(Me)(Me); R′ = H

V: R = H; R′ = —CH_2—CH(O)—C(Me)(Me)

VII: R = H; R′ = —CH_2—CHOH—C(OH)(Me)(Me)

Confirmation of the structure IV for sibiricin has been provided by alkaline hydrolysis, when sibiricic acid VIII was obtained. This acid has mol. formula $C_{16}H_{20}O_6$ and m.p. 185–186°. It does not give a positive ferric chloride test, but has properties of a free carboxylic acid. In UV light it does not give any fluorescence. Its spectral data (Experimental) indicates that the epoxide function and coumarin moiety have undergone change to form a new structure. This change involves the conversion of the coumarin into *o*-hydroxycinnamic acid derivative IX, followed by intramolecular reaction between the phenolic and the epoxide grouping forming a new ring. Such a transformation is well known in the coumarin series.[8]



IV → IX → VIII

The NMR spectrum of sibiricin (Fig. I) is in full agreement with the structure assigned. A pair of doublets, at τ 2·05 (1H) and τ 3·90 (1H), J = 9·5 c/s) correspond to protons at 4 and 3-positions. Two singlets at τ 3·65 (1H) and τ 6·08 (6H) can be

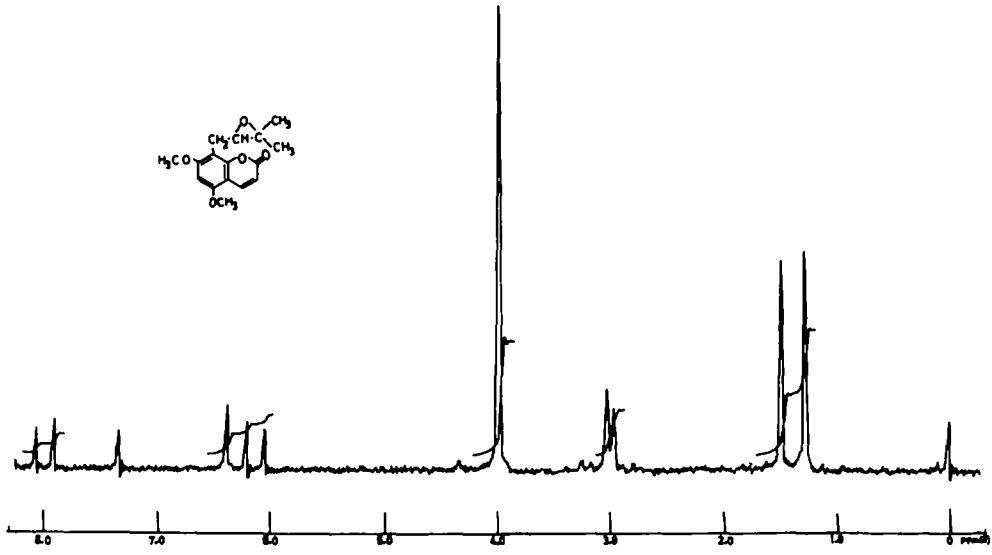
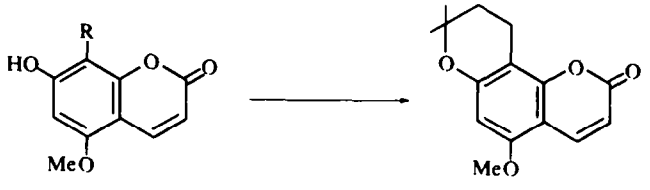


FIG. 1 NMR Spectrum.

assigned to the proton at position 6 and two OMe groups at 5 and 7 positions respectively. Another pair of singlets at τ 8·35 and τ 8·75 (3H each) are attributable to the two Me's of the tertiary C atom of the side chain. Other protons of the C-5 residue show a doublet centred at τ 7·02.

The structure of sibiricin has been confirmed by synthesis. A possible route would involve nuclear allylation of 7-hydroxy-5-methoxycoumarin X providing the 8-γ,γ-dimethylallyl derivative XI as intermediate. The reaction, however, does not proceed satisfactorily under the conditions reported in a similar case.[9] The properties and spectral studies of a product obtained in poor yield reveal that the nuclear allylation is followed by cyclization to give the dimethylchroman XII (5-methoxy-dihydro seselin).

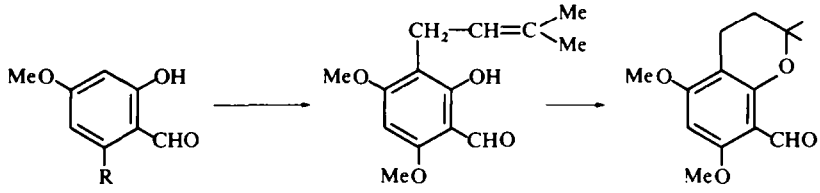


X: R = H XII

XI: R = $-CH_2-CH=C(Me)(Me)$

A different approach has, therefore, been adopted. Spath *et al.*,[10] in connection with the synthesis of osthol, recorded that the sodium salt of 2-hydroxy-4-methoxybenzaldehyde XIII could be allylated in the 3-position. Following these conditions, 2-hydroxy-4,6-dimethoxybenzaldehyde XIV as its sodium salt, was refluxed with γ,γ-dimethylallyl bromide to give the 3-dimethylallyl derivative XV. Nuclear allylation

only at the 3-position was shown by converting the product into the corresponding 2,2-dimethylchroman derivative XVI by treatment with formic acid.

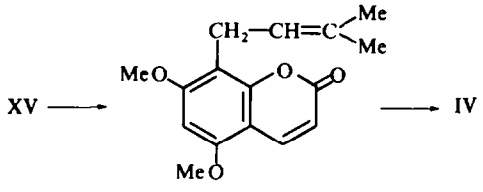


XIII; R = H
XIV: R = OMe

XV

XVI

Perkin condensation of XV gave 5,7-dimethoxy-8-dimethylallylcoumarin XVII, which was subsequently epoxidized with perbenzoic acid to yield (±) sibiricin IV. The synthetic sample agreed fully in m.p., (m.m.p. undepressed), analytical values, UV and IR spectra with the natural sample. There is, however, a difference in the NMR spectrum of the synthetic and natural specimens of sibiricin. The three protons of the side chain at the 8 position (—$\underline{CH_2}$—$\underline{CH}$—C:$(CH_3)_2$) show a doublet at τ 7·02 in the natural sample whereas in the synthetic sample this doublet is not very sharp, though it is observed in the same region. Other signals in both the spectra are identical. This difference is attributable to the difference in stereochemistry, the natural sibiricin being dextro- and the synthetic being racemic.



XVII

EXPERIMENTAL

All m.ps are uncorrected. Pet. ether had b.p. 40–60°. UV spectra were taken on a Perkin–Elmer spectrophotometer in 95% EtOH; log ε values are given in brackets. IR spectra were recorded in KBr on a Perkin–Elmer infracord. The NMR spectra were taken in $CDCl_3$ soln with TMS as internal standard on Varian A-60 model and the signals are reported in τ values.

Extraction of umbels

(i) *Pet. ether extraction* (osthol, imperatorin, bergapten and sibiricin). The air dried umbels (1·5 kg) obtained from Gulmarg (Kashmir), were finely powdered and extracted with pet. ether. The solvent was removed and the solvent free extract was chromatographed on acetic acid deactivated alumina and eluted successively with pet. ether, pet. ether–benzene (3:1), pet. ether–benzene (1:1), benzene, benzene–ether (1:1), ether and chloroform.

The fraction (9·0 g) eluted with pet. ether–benzene (3:1) after several crystallizations from MeOH had m.p. 83–84°. Its blue fluorescence in UV light and R_f value on TLC agreed with an authentic sample of osthol; mixed m.p. was undepressed.

The first few fractions of pet. ether–benzene (1:1), yielded a yellow crystalline substance (1·2 g) which after repeated crystallization from MeOH became colourless, m.p. 100–102°, green fluorescence in UV light. It was identified as imperatorin by mixed m.p. and by TLC comparison with an authentic sample.

The last ether–benzene (1:1) fractions yielded (0·2 g) which crystallized from MeOH as colourless

crystals, m.p. 188–189°. It was identified as bergapten by TLC and fluorescence in UV light with an authentic sample, mixed m.p. was undepressed.

The fractions eluted with ether yielded (2 g) a white powder, which on recrystallization from EtOAc and then from MeOH was obtained as silky needles, m.p. 152–153°; TLC (silica gel G; solvent system; isopropyl ether, n-heptane 70:30) gave a single spot which had blue fluorescence in UV light. It has been named sibiricin. It was soluble in alcohol, ether and chloroform, and insoluble in warm 5% Na_2CO_3 aq and 5% NaOH aq. In alcoholic alkali it dissolved to a yellow soln. (Found: C, 65·9; H, 6·2; MeO, 18·2. $C_{16}H_{18}O_5$ requires: C, 66·2; H, 6·2; $2CH_3O$, 21·4%); $[\alpha]_D^{30°} = +60·4°$ (*c*, 3·31 dioxan). λ_{max} 240 (3·76); 252 sh (3·92), 259 sh (3·98) and 327 mμ (4·14); λ_{min} 233 (3·73) and 270 (3·07); ν_{max}^{KBr} 2967, 2841, 1740, 1613, 1580 sh, 1511, 1481, 1466, 1441, 1389, 1333, 1290, 1250, 1236, 1212, 1186, 1140, 1117, 1070, 1044, 1033, 990, 952, 901, 846, 833, 817 and 781 cm^{-1}.

Pet. ether, benzene and chloroform did not elute any crystallizable material.

Sibiricin glycol. Sibiricin (0·1 g) and 1% aq. oxalic acid (50 ml) were refluxed for 1 hr, the clear soln cooled and treated with $NaHCO_3$. The white ppt was filtered and crystallized repeatedly from EtOAc, m.p. 185–186°; TLC gave a single spot with brilliant green fluorescence in UV light. (Found: C, 62·1; H, 6·4; $C_{16}H_{20}O_6$ requires: C, 62·3; H, 6·5%); $[\alpha]_D^{27°} = +27·1°$ (*c*, 1·165, dioxan); λ_{max} 261 (3·95) and 326 mμ (4·07), λ_{min} 236 (3·72 and 274 mμ (3·06); ν_{max} (Nujol), 3448, 2941, 2874, 1725, 1600, 1489, 1449, 1433, 1370, 1319, 1250, 1227, 1205, 1173, 1160, 1136, 1117, 1099, 1042, 990, 967, 943, 897, 856, 816 and 794 cm^{-1}.

Hydrolysis of sibiricin. Sibiricin (0·1 g) and 10% NaOH aq (25 ml) were refluxed for 4 hr. The soln remained clear on cooling. On acidification with HCl a white solid was obtained. It melted at 185–186° (dec), unchanged by further crystallization from EtOAc and depressed by sibiricin glycol. It dissolved in ether and in water giving acid reaction and no ferric reaction. (Found: C, 61·8; H, 6·3. $C_{16}H_{20}O_6$ requires: C, 62·3; H, 6·5%); $[\alpha]_D^{25°} = -70°$ (*c*, 0·512, dioxan); λ_{max} 205 (4·58) and 306 mμ (4·12), λ_{min} 260 mμ (3·55); ν_{max}^{KBr} 3448, 2985, 1703, 1627, 1508, 1417, 1360, 1333, 1302, 1209, 1177, 1152, 1126, 1101, 1055, 1000, 980, 962, 947, 881, 886, 858, 823, 792 and 769 cm^{-1}.

(ii) *Benzene extract* (osthol). After pet. ether extraction the residual umbels were extracted with benzene and the benzene extract (20·7 g) was chromatographed on AcOH deactivated alumina (400 g) using pet. ether, benzene, $CHCl_3$ and EtOH as successive eluents. Only benzene fractions yielded a crystalline solid (0·45 g), which after several crystallizations from MeOH had m.p. 83–84°, undepressed by authentic osthol.

Ethanolic extract (D-mannitol). The residual umbels (1·5 kg) were extracted with alcohol and the concentrated extract, kept overnight in the cold, yielded a dark brown solid mass. It was separated and washed with MeOH whereby it turned into colourless cyrstals. On recrystallization from EtOH it melted at 165–166°. It was freely soluble in water, sparingly in EtOH and insoluble in ether. The mixed m.p. with the authentic sample of D-mannitol showed no depression. The hexa-acetate prepared by refluxing with Ac_2O and NaOEt crystallized from MeOH, m.p. 123°, agreeing with mannitol hexa-acetate.

Reaction of 7-hydroxy-5-methoxy coumarin (X) *with γ,γ,-dimethylallyl bromide.* 7-Hydroxy-5-methoxy-coumarin (0·66 g) in acetone (20 ml) was treated with NaOH aq (0·15 g in 3 ml H_2O) at 0°. The yellow Na-salt was filtered off, dried in vacuum, suspended in anhyd benzene (20 ml) and refluxed with γ,γ-dimethylallylbromide (2·1 ml) with continuous stirring. The reaction mixture was cooled, acidified with ice cold 0·1N HCl and extracted with ether (3 × 50 ml). The combined ether extract was washed with 0·1N Na_2CO_3 (3 × 20 ml), which when acidified gave unchanged starting material (0·5 g), m.p. 244–245°. The neutral ethereal fraction on evaporation gave a dark coloured syrup, which on chromatography on silica gel (10 g) gave colourless prisms (0·035 g), m.p. 175–176°, (Found: C, 68·5; H, 6·0; $C_{15}H_{16}O_4$ requires: C, 69·2; H, 6·2%); NMR spectrum showed a pair of doublets at τ 1·95 (1H) and τ 3·82 (1H), J = 10 c/s assigned to protons at 4 and 3 positions, two singlets at τ 3·72 (1H) and τ 6·1 (3H) attributed to a proton at 6 position and a OMe group at 5 position respectively, a pair of triplets at τ 7·15 (2H) and τ 8·05 (2H), J = 7 c/s, assigned to two adjacent methylene groups at 3' and 4'-positions of the chroman ring, a singlet at τ 8·62 (6H) due to *gem* dimethyl grouping at 2'-position.

3-γ,γ-Dimethylallyl-2-hydroxy-4,6-dimethoxybenzaldehyde (XV). 2-Hydroxy-4,6-dimethoxybenzaldehyde (5·0 g) in benzene (30 ml) was treated with NaOMe (0·07 g in 16 ml MeOH), kept in the refrigerator for ½ hr, and the white Na-salt was filtered off and dried in vacuum. It was suspended in anhyd benzene (125 ml) and treated with γ,γ-dimethylallylbromide (17 ml) with stirring and the product was chromatographed on silica gel (100 g). A mixture of two components was obtained when eluted with pet. ether. Further separation was affected by fractional crystallization from alcohol yielding first 2-hydroxy-4,6-dimethoxybenzaldehyde (3·8 g), m.p. 70–71°, and then 3-γ,γ-dimethylallyl-2-hydroxy-4,6-dimethoxy-benzaldehyde (0·95 g) m.p. 110–111°. (Found: C, 66·9; H, 7·3; $C_{14}H_{18}O_4$ requires: C, 67·2; H, 7·3%);

NMR a singlet at τ 4·05 (1H) assigned to proton at 5 position; two singlets at τ 6·1 (3H) and τ 6·13 (3H) attributable to OMe's at 4 and 6 positions: signals at τ 6·75 (doublet; 2H, J = 7 c/s), τ 4·8 (triplet; 1H, J = 7 c/s) τ 8·25 (singlet; 3H) and τ 8·32 (singlet; 3H) due to the side chain (—CH_2—CH=C$\begin{smallmatrix}Me\\Me\end{smallmatrix}$) at 3-position.

8-Formyl-5,7-dimethoxy-2,2-dimethylchroman (XVI). 2-Hydroxy-3-dimethylallyl-4,6-dimethoxybenzaldehyde (0·07 g) was heated at 100° with formic acid (1·5 ml; 95%) for 2 hr. The reaction mixture was diluted with water (100 ml) and extracted with ether. The ether extract was washed with water dried over Na_2SO_4 and evaporated to dryness. The residual syrup was chromatographed on silica gel (10 g), when pet. ether eluate gave unchanged starting material (0·008 g), m.p. 106–109°. Benzene eluted 8-formyl-5,7-dimethoxy-2,2-dimethylchroman (0·04 g), m.p. 105–107°. It crystallized from EtOAc–ether as yellow needles. (Found: C, 66·8; H, 7·0. $C_{14}H_{18}O_4$ requires: C, 67·2; H, 7·3%); NMR spectrum in CCl_4: a singlet at τ 4·0 (1H) can be assigned to proton at the 6 position, two singlets at τ 6·13 (3H) and τ 6·19 (3H) attributed to two OMe's at 5 and 7 positions, a pair of triplets at τ 7·47 (2H) and τ 8·27 (2H), J = 7 c/s due to two adjacent methylene groups at 3' and 4'-position of the chroman ring; a singlet at τ 8·65 (6H) due to *gem* dimethyl grouping at 2'-position; a signal at τ −0·4 due to an aldehyde group.

Perkin condensation of 2-hydroxy-3-γ,γ-dimethylallyl-4,6-dimethoxybenzaldehyde (XV). Compound XV (0·4 g) was dissolved in Ac_2O (15 ml) and the soln refluxed with fused NaOEt (8 g) at 170–180° for 20 hr. The mixture was cooled and poured on crushed ice, when a dark brown solid separated. On TLC it showed 3 spots, one of which corresponded to the starting material. The crystallization of the crude mixture from alcohol yielded 2-acetoxy-3-γ,γ-dimethylallyl-4,6-dimethoxy-benzaldehyde, (0·31 g) which crystallized from alcohol as colourless prisms m.p. 133–134°. (Found: C, 66·1; H, 6·9; $C_{16}H_{20}O_5$ requires: C, 65·7; H, 6·9%); NMR spectrum was essentially similar to that of 2-hydroxy-3-γ,γ-dimethylallyl-4,6-dimethoxybenzaldehyde except for a signal for acetoxyl at τ 7·63 (3H).

The mother liquor showed the presence of two components on TLC. Column chromatography on silica gel (10 g) yielded the starting material (0·012 g), m.p. 106–107° in pet. ether fraction. Further elution with pet. ether–benzene (1:1) gave the required coumarin (XVII) which crystallized from alcohol as colourless needles (0·06 g), m.p. 156–158°. (Found: C, 70·4; G, 7·0. $C_{16}H_{18}O_4$ requires: C, 70·1; H, 6·6%); NMR a pair of doublets at τ 1·95 (1H) and τ 3·83 (1H), J = 10 c/s assigned to protons at 4 and 3-positions; singlets at τ 3·55 (1H) and τ 6·05 (6H) due to a proton at the 6-position and protons of two OMe's at 5 and 7 positions respectively, signals at τ 6·53 doublet; 2H, J = 7 c/s) τ 4·63 (triplet; 1H, 7 c/s and two singlets at 8·15 and τ 8·42 (3H each) due to the side chain (—CH_2—CH=C$\begin{smallmatrix}Me\\Me\end{smallmatrix}$).

Epoxidation. A soln of XVII (0·03 g) in $CHCl_3$ (3 ml) was added to ice cold soln of perbenzoic acid (0·016 g) in $CHCl_3$ (2 ml) and the reaction mixture was kept in a refrigerator for 2 days. The excess of perbenzoic acid was washed out with Na_2CO_3 (2 × 3 ml) and finally with H_2O (2 × 5 ml). The $CHCl_3$ layer was dried (Na_2SO_4) and evaporated to dryness yielding the epoxide of the coumarin (0·03 g). This crystallized from EtOAc as colourless silky needles m.p. 152–153°; NMR spectrum a pair of doublets at τ 2·05 (1H) and τ 3·99 (1H) J = 10 c/s assigned to protons at 4 and 3 positions, two singlets at τ 3·65 (1H) and τ 6·05 (6H) due to a proton at 6 position and protons of two OMe groups at 5:7 positions; signals at τ 7·05 (triplet; 3H) and two singlets at τ 8·55 (3H) and τ 8·73 (3H) attributable to the side chain (—CH_2—CH$\begin{smallmatrix}O\\\diagup\diagdown\\C\end{smallmatrix}\begin{smallmatrix}Me\\Me\end{smallmatrix}$). The synthetic sample agreed to TLC behaviour, UV, IR and analytical value with the natural specimen.

Acknowledgements—The author's thanks are due to Dr. M. W. Klohs, Riker Laboratory, California, U.S.A. for NMR spectrum of natural sample of sibiricin and Mr. Y. K. Sarin of Jammu Regional Research Laboratory for supply of plant material.

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Recently Chakraborty and Chowdhury (Tet. Letters 1967, 3471) have given for mexoticin from *Murraya exotica*, the structure of sibiricin glycol (IX). The properties of these two are similar. They could not be directly compared with regard to C=O frequency and rotation for want of the sample of mexoticin. However, sibiricin glycol gave $[\alpha]_D^{24°}$, 41·0° (*c*, 0·8, $CHCl_3$).