Structure of a new anticomplementary dihydroxycinnamate-derived polymer from *Symphytum asperum* (Boraginaceae)

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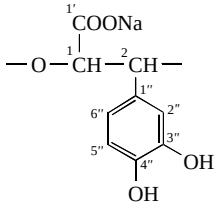
A water-soluble high-molecular preparation with strong anticomplementary and antioxidative activity isolated from the roots of *Symphytum asperum* is principally polyoxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene.

A crude glucofructan preparation isolated[1] from the roots of *Symphytum asperum* (Boraginaceae) displayed anticomplementary and radical-scavenging activity, in contrast to other water-soluble plant polysaccharides.[2] The activity-guided fractionation[2] (ultrafiltration in an Amicon chamber using membranes with cut-off values of 1000 kDa followed by fractional dissolution of a high-molecular component in a veronal–saline buffer, pH 7.35, and gel-permeation chromatography on a column with Sepharose 2B) led to the elimination of more than 90% of the polysaccharide material and to the isolation of a water-soluble high-molecular (>1000 kDa) HM-SA† fraction (as the Na salt) having minor carbohydrate (25.7%) and protein (1.07% N) content. Its UV spectrum contained three absorption bands at 252 (moderate), 282 (strong) and 286 (strong) nm. According to these data, the active principle of the preparation was regarded as a high-molecular phenolic compound.[3] In the present report, the structure of the major building unit of HM-SA was elucidated using IR and NMR spectroscopy.

The IR spectrum of HM-SA (recorded in a KBr pellet using a Perkin-Elmer 571 spectrophotometer) contained absorption bands typical of phenol-carboxylic acids[4] at 3420 (OH), 2930 (CH), 1620 (ionised carboxyl), 1600, 1510, 1450 (aromatic C=C), 1410, 1220 (phenols), 1270, 1130, 1075, 1030 (R–O–R′), 880 (aromatic C–H, one isolated hydrogen atom), and 830 (aromatic C–H, two neighbouring hydrogen atoms) cm^{-1}.

The ^{13}C NMR spectrum of HM-SA contained nine signals,‡ five of them belonging to CH and four, to non-protonated carbons, as revealed by the APT data.[5] According to the chemical shift values, two signals (78.2 and 80.4 ppm) were attributed to aliphatic protonated carbons linked to oxygen, six signals were ascribed to aromatic carbons (protonated ones at 117.4, 118.6 and 122.3 ppm, and non-protonated ones at 131.5, 143.8 and 144.6 ppm), and the last broadened signal (175.4 ppm) was attributed to a carboxyl.

The ^1H NMR spectrum of HM-SA contained four signals at 4.88, 5.33, 7.13, and 7.24 ppm, one of them (7.13 ppm) being of double intensity. Unfortunately, the spin–spin coupling constants could not be determined due to broadening of these signals. The two-dimensional heteronuclear $^1H/^{13}C$ HSQC spectrum showed correlations between protons and carbons having chemical shifts of 4.88/80.4, 5.33/78.2, 7.13/118.6, 7.13/122.3, and 7.24/117.4 ppm. According to these data, the backbone of the substance is a polyether chain similar to that of the ethylene oxide polymers, two carbon atoms of every repeating unit being regularly substituted by dihydroxyphenyl and carboxyl groups, respectively. Otherwise, the repeating unit of the polymer may be represented as an α-hydroxy-β-(dihydroxyphenyl)propionic acid residue, and hence, the polyether is polyoxy-1-carboxy-2-(dihydroxyphenyl)ethylene. The presence of two hydroxy groups in the *meta*- and *para*-positions of an aromatic ring was unambiguously proved by a 1D NOE experiment, performed in the difference mode. Pre-irradiation of the proton with a chemical shift of 5.33 ppm provided a NOE for two aromatic protons with chemical shifts of 7.13 (3%) and 7.24 (1%) ppm. Thus, both of the *ortho*-positions in the dihydroxyphenyl ring were occupied by protons. The different values of NOE for these two protons, as well as their different chemical shifts in the ^1H NMR spectrum and different positions of resonances of the corresponding carbons in the ^{13}C NMR spectrum, excluded the possibility of symmetrical bis-*meta*-substitution of the aromatic ring by two hydroxy groups.



Different phenylpropanoids are well-known constituent units of structural polymers of plant cell walls, lignols being the main constituents of lignin,[6] whereas arylpropionic acids are abundant in the aromatic part of suberin.[7] Both lignin and suberin are high-molecular three-dimensional cross-linked polymers, which are practically insoluble in water. Their structural analysis is based mainly on the data of chemical degradations.[8,9] Partial characterization of these polymers can be performed using ^{13}C NMR spectra obtained in the solid state.[10,11] In contrast, HM-SA may be regarded as a linear polymer of 2,3-epoxy-3-(3,4-dihydroxyphenyl)propionic acid. It is readily soluble in water like many mucilaginous plant polysaccharides. Thus, it is impossible to compare directly the ^{13}C NMR spectrum of HM-SA with the published spectra of insoluble arylpropanoid polymers, but the attribution of signals to related carbons in the aromatic and aliphatic moieties of lignin,[10] suberin,[11] model compounds[12] and HM-SA is not controversial.

It may be concluded that the high-molecular water-soluble compound with strong anti-complementary activity, isolated from the roots of *S. asperum*, represents a new class of natural polyethers. Its formation could be represented chemically as double bond epoxidation in a dihydroxycinnamate precursor followed by polymerization of the resulting oxirane.

† High-molecular preparation from *Symphytum asperum*.

‡ NMR spectra were recorded using a Bruker DRX-500 spectrometer for a 1% solution of the polymer in D_2O at 70 °C with acetone (2.225 ppm for ^1H and 31.45 ppm for ^{13}C) as an internal standard. Pre-irradiation time for the 1D NOE experiment was 1 s, the signal of pre-irradiated proton in the difference spectrum was taken as 100%. Two-dimensional HSQC spectrum was obtained using a standard pulse sequence from the Bruker software.

^1H NMR, δ: 7.24 (H-2″), 7.13 (H-5″ and H-6″), 5.33 (H-1), 4.88 (H-2).

^{13}C NMR, δ: 175.4 (C-1′), 144.6 (C-3″), 143.8 (C-4″), 131.5 (C-1″), 122.3 (C-6″), 118.6 (C-5″), 117.4 (C-2″), 80.4 (C-2), 78.2 (C-1).

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