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Aucuparin and Methoxyaucuparin, Two Phenolic Biphenyl Derivatives from the Heartwood of *Sorbus aucuparia* (L.) *

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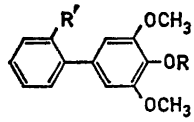
Dedicated to Professor *Hans von Euler* on his ninetieth birthday.

The investigation is described of two phenolic biphenyl derivatives, aucuparin (1a) (4-hydroxy-3,5-dimethoxybiphenyl) and methoxyaucuparin (1b) (4-hydroxy-3,5,2′-trimethoxybiphenyl), from the heartwood of *Sorbus aucuparia* (L.). The structures of these compounds follow from the results of oxidative degradation experiments, from their spectral properties and from the syntheses of the corresponding methyl ethers.

The isolation of aucuparin and methoxyaucuparin from the heartwood of *Sorbus aucuparia* (L.), mountain ash, (Rosaceae, Pomoideae), has recently been reported [1]. Full details of the work leading to the structures (1a) and (1b) for these novel phenolic substances are now presented.

Aucuparin and methoxyaucuparin have the compositions $C_{12}H_7(OH)(OCH_3)_2$ and $C_{12}H_6(OH)(OCH_3)_3$, respectively. On methylation both compounds gave the corresponding methyl ethers (1c) and (1d). The aucuparins gave no characteristic colour reaction with bisdiazotised benzidine which indicates that the *o*- and *p*-positions to the hydroxyl groups are blocked. The ferric chloride reaction [2] was similar to that given by several pyrogallol derivatives. The compounds did not give any colour reaction with vanillin and concentrated sulphuric acid (test for phloroglucinol groups [3]). On oxidation with permanganate aucuparin furnished benzoic acid and methoxyaucuparin 2-methoxybenzoic acid. These results and the composition of the aucuparins

* Preliminary communication *cf.* Ref.[1]



1a: R = R' = H
1b: R = H; R' = OCH_3
1c: R = CH_3; R' = H
1d: R = CH_3; R' = OCH_3

indicated that they might be biphenyl derivatives, aucuparin possessing an unsubstituted and methoxyaucuparin an *ortho* methoxylated phenyl group.

The ultraviolet absorption of the aucuparins furnished independent indications as to the nature of these compounds. The absorption curve of aucuparin in 95 % ethanol had one maximum (at 273 mμ, ε 14 100) as expected for a biphenyl derivative carrying auxochromic substituents in the 3- and 4-positions in one ring but none in the 2-positions or in the other ring. Methoxyaucuparin in the same solvent showed two absorption maxima of lower intensity (265 mμ, ε 7660 and 286 mμ, ε 6100), indicating the presence of a new, relatively strong chromophore, as well as a decrease in the conjugation in the biphenyl system. The two effects are well explained by the methoxyl group present in the 2'-position [4]. A methoxyl group located in 2-, 3'- or 4'-position would not be expected to give rise to both of those effects.

It appeared possible that aucuparin might be 3,5-dimethoxy-4-hydroxybiphenyl and methoxyaucuparin the corresponding 2'-methoxybiphenyl. 3,4,5-Trimethoxybiphenyl (1 c) was therefore synthesised according to the method developed by Erdtman, Haglid and Stjernström for the synthesis of unsymmetrical biphenyl derivatives [5]. Ullmann coupling of 3,4,5-trimethoxyiodobenzene [6] and methyl-*o*-bromobenzoate furnished a reaction product which was subjected to alkaline hydrolysis. The dicarboxylic acids obtained were easily separated and the 2'-carboxy-3,4,5-trimethoxybiphenyl decarboxylated to 3,4,5-trimethoxybiphenyl (1c) which was found to be identical with aucuparin methyl ether. The same compound was also obtained albeit in poor yield by mixed Ullmann coupling of iodobenzene and 3,4,5-trimethoxyiodobenzene.

Similarly 3,4,5,2'-tetramethoxybiphenyl (1d) was prepared using methyl 3-iodo-4-methoxybenzoate [7] and 3,4,5-trimethoxyiodobenzene. It was identical with methoxyaucuparin methyl ether.

The syntheses of these two methyl ethers settled the carbon skeleton and the substitution patterns of the aucuparins.

The nuclear magnetic resonance spectra of aucuparin and methoxyaucuparin are shown in Fig. 1. The signals at about 5.5 ppm occurring in both spectra are found to be shifted towards lower applied fields if a trace of trifluoroacetic acid is added to the solutions. These signals can accordingly be assigned to the phenolic hydroxyl protons.

The signals at about 3.80—3.90 ppm, found in both spectra, are assigned to the protons of the methoxyl groups. The integrated intensity of the signal at 3.88 ppm in the spectrum of aucuparin corresponds to two methoxyl groups whilst the signals at 3.80 and 3.89 ppm in the spectrum of methoxyaucuparin correspond to one and two methoxyl groups, respectively.

The spectrum of aucuparin exhibits a signal group around 7.4 ppm with an integrated intensity corresponding to five protons. This group is assigned to the protons of the phenyl group. The resonance positions agree well with

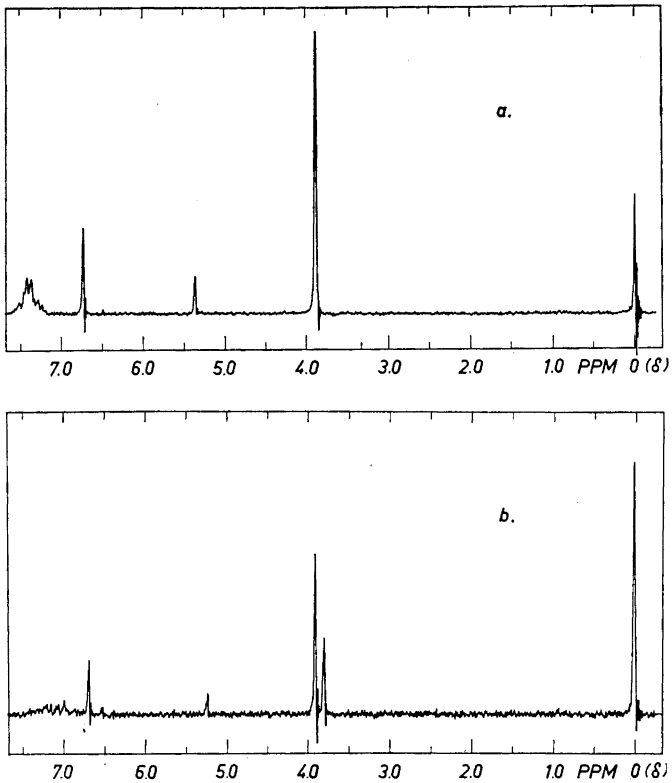


Fig. 1. Nuclear magnetic resonance spectra of aucuparin (a) and methoxyaucuparin (b). (Ca. 10—15 % carbon tetrachloride solutions; tetramethylsilane as internal standard; Varian A 60 instrument operating at 60 Mc/s).

those observed for protons on unsubstituted biphenyl rings. Similarly the spectrum of methoxyaucuparin exhibits a highly split signal centered around 7.1 ppm with an integrated intensity corresponding to four protons, assigned to the aromatic protons of the *ortho* methoxylated phenyl group.

The sharp signal at 6.73 ppm in the spectrum of aucuparin, and at 6.68 ppm in that of methoxyaucuparin, corresponds to two protons. This signal is assigned to the two aromatic hydrogens on the 3,4,5-trisubstituted phenyl group common to both aucuparins. The resonance fields of these two aromatic hydrogens are identical within the resolution of the spectrometer. The resonance positions of the protons of the two methoxyl groups on this phenyl ring are similarly identical (aucuparin, 3.88 ppm; methoxyaucuparin, 3.89 ppm). This strongly suggests that this phenyl group is symmetrically substituted with the hydroxyl group in the 4-position. The NMR results are thus consistent with the structures (1a) and (1b) for aucuparin and methoxyaucuparin, respectively.

The syntheses of the aucuparins will be described in the following publication.

EXPERIMENTAL

Melting points, taken on a Kofler micro hot stage, are uncorrected. The ultraviolet absorptions were determined in alcohol (95 %) solutions on a Beckman DK 2 spectrophotometer. The nuclear magnetic resonance (NMR) spectra were recorded in ca. 10—15 % carbon tetrachloride solutions on a Varian A 60 instrument operating at a fixed frequency of 60 Mc/s. Tetramethylsilane was used as internal reference. The shifts are given in δ-units (ppm).

The isolation of aucuparin and methoxyaucuparin will be described elsewhere.

Aucuparin (1a). M.p. 101—101.5° (sublimed under reduced pressure); λ_{max} 273 mμ (ε 14 100); NMR spectrum see Fig. 1a. (Found: C 72.9; H 6.1; OCH_3 27.3. $C_{12}H_8O(OCH_3)_2$ requires C 73.0; H 6.1; OCH_3 26.8 %).

Aucuparin methyl ether (1c) was obtained by treatment of aucuparin with excess diazomethane in ether containing some methanol, or by using dimethylsulphate as methylating agent. Because of the poor solubility of the alkali salts of the aucuparins, sodium hydroxide (5.4 ml, 2 N) was added dropwise to the stirred solution of the aucuparin (1.0 g) in methanol (2 ml) and dimethylsulphate (0.7 ml) on a water bath. The compound thus obtained had, after recrystallisation from methanol-water and sublimation under reduced pressure, m.p. 88—89° and λ_{max} 263 mμ (ε 17 600). (Found: C 73.1; H 6.6; OCH_3 37.3. $C_{13}H_7(OCH_3)_3$ requires C 73.8; H 6.6; OCH_3 38.1.)

Acetylation of aucuparin with acetic anhydride in pyridine gave *aucuparin acetate*, m.p. 150—151° (plates from ethanol); λ_{max} 258 mμ (ε 15 700). (Found: C 70.5; H 5.9; OCH_3 22.5; $COCH_3$ 15.4. $C_{12}H_7(OCH_3)_2(OCOCH_3)$ requires C 70.6; H 5.9; OCH_3 22.8; $COCH_3$ 15.8).

Benzoylation of aucuparin with benzoyl chloride in pyridine gave *aucuparin benzoate*, m.p. 152.0—153.5° (needles from ethanol). (Found: C 75.7; H 5.4; OCH_3 18.5. $C_{19}H_{12}O_2(OCH_3)_2$ requires C 75.4; H 5.4; OCH_3 18.5).

Methoxyaucuparin (1b). M.p. 120—122°; λ_{max} 265 mμ (ε 6770) and 286 mμ (ε 6100); NMR spectrum see Fig. 1b. (Found: C 69.1; H 6.4; OCH_3 35.3. $C_{12}H_7O(OCH_3)_3$ requires C 69.2; H 6.2; OCH_3 35.7).

Methoxyaucuparin methyl ether (1d) was obtained by methylation with diazomethane or dimethyl sulphate as described for aucuparin methyl ether (1c) and had m.p. 71.5—72.0°, after recrystallisation from methanol-water and sublimation under reduced pressure; λ_{max} 256 mμ (ε 9940) and 285 mμ (ε 6600). (Found: C 69.8; H 6.6, OCH_3 44.1. $C_{12}H_6(OCH_3)_4$ requires C 70.1; H 6.6; OCH_3 45.2).

Methoxyaucuparin with acetic anhydride in pyridine gave *methoxyaucuparin acetate* which after recrystallisation from ethanol and sublimation under reduced pressure had m.p. 119—120°; λ_{max} 264 mμ (ε 13 200) and 285 mμ (ε 11750). (Found: C 67.7; H 6.1; $COCH_3$ 13.7. $C_{12}H_6(OCH_3)_3(OCOCH_3)$ requires C 67.5; H 6.0; $COCH_3$ 14.2.)

Methoxyaucuparin benzoate was prepared from methoxyaucuparin and benzoylchloride in pyridine and had m.p. 191.0—193.5° after recrystallisation from benzene. (Found: C 72.9; H 5.6; OCH_3 24.6. $C_{19}H_{11}O_2(OCH_3)_3$ requires C 72.5; H 5.5; OCH_3 25.5).

Colour tests. Aucuparin and methoxyaucuparin gave a pale blue colour reaction with anhydrous ferric chloride in chloroform. On addition of pyridine the colour changed to dark blue [2]. The reaction was very similar to that given by pyrogallol, pyrogallol 1,3-dimethyl ether and ethyl-3,4,5-trihydroxybenzene. Phloroglucinol and methylphloroglucinol gave no colour reaction with this reagent. Aucuparin and methoxyaucuparin gave no colour reaction with vanillin in sulphuric acid (70 %), characteristic of the phloroglucinol grouping [3]. The aucuparins gave no definite colour reaction with bisdiazotised benzidine.

Oxidation of aucuparin and methoxyaucuparin with permanganate. Aucuparin (or methoxyaucuparin) (0.5 g) was suspended in an aqueous solution of sodium carbonate (20 ml; 5 %) which was kept at about 50° whilst vigorously stirred. Potassium permanganate (5 g) was added in small portions during 12 h. Addition of an aqueous sodium bisulphite solution and acidification gave an almost clear solution which was extracted with ether. The ether extract was shaken with saturated aqueous bicarbonate solution and the bicarbonate extract was acidified and re-extracted with ether. After evaporation of the ether a crude crystalline product was obtained which was purified by sublimation under reduced pressure. Aucuparin thus gave benzoic acid (0.086 g) and methoxyaucuparin 2-methoxybenzoic acid (0.050 g).

The two aucuparin methyl ethers (1c) and (1d) were very resistant to permanganate oxidations. The yield of acidic products obtained after prolonged treatment at 100° was very poor and no trimethoxygallic acid could be isolated from these oxidations.

Demethylation of aucuparin and methoxyaucuparin. Aucuparin (or methoxyaucuparin) (0.35 g) in hydrobromic acid (25 ml; 48 %) was heated under reflux in an atmosphere of oxygen free nitrogen for 1.5 h. Water (25 ml) was added and the reaction mixture allowed to cool. The non-fluorescent demethylated product crystallised. Recrystallisation from aqueous methanol and sublimation under reduced pressure gave the pure product.

Aucuparin thus gave *3,4,5-trihydroxybiphenyl* (0.29 g) which had m.p. 196—198° and λ_{max} 272 mμ (ε 13 500). (Found: C 71.0; H 5.2. $C_{12}H_{10}O_3$ requires C 71.3; H 5.0).

Methoxyaucuparin gave *3,4,5,2'-tetrahydroxybiphenyl*, which because of its water solubility was isolated by extraction with ether. The non-fluorescent product (0.22 g) had m.p. 150.0—151.5°, and λ_{max} 262 mμ (ε 7720) and 290 mμ (ε 6740). (Found: C 66.0; H 4.7. $C_{12}H_{10}O_4$ requires C 66.1; H 4.6). Prolonged (14 h) boiling of methoxyaucuparin with hydrobromic acid gave the same tetrahydroxybiphenyl in the same yield. There were no indications of the formation of a dibenzofuran derivative (no fluorescense in ultraviolet light).

3,4,5-Trimethoxyiodobenzene [6]. 3,4,5-Trimethoxyaniline, m.p. 110—112° (lit.[6] 113—114°), was prepared in high yield (92 %) by the catalytic hydrogenation of 3,4,5-trimethoxynitrobenzene [8] in ethanol using a palladium charcoal catalyst (10 %).

Sodium nitrite solution (37.6 g in 20 ml of water) was added drop by drop to a stirred and ice-cooled solution of the 3,4,5-trimethoxyaniline (10 g) in a mixture of water (35 ml) and concentrated sulphuric acid (8 ml). The yellow reaction mixture was poured into a stirred potassium iodide solution (14 g in 30 ml of water) kept at 50°. When the decomposition of the diazonium salt was complete, the reaction mixture was allowed to cool and extracted with ether. Evaporation of the dried (Na_2SO_4) ether solution gave crude 3,4,5-trimethoxyiodobenzene (13.5 g) which after recrystallisation from ethanol had m.p. 85—87° (lit[6]. 82—83°).

Ullmann couplings. (a) Iodobenzene (4.9 g) and 3,4,5-trimethoxyiodobenzene (1.0 g) were thoroughly mixed with copper bronze (6.9 g) in a pyrex tube, which was then sealed, and heated in a sand bath at 240° for 1 h. The cooled reaction mixture was leached with methanol. After evaporation of the solvent, the residue was distilled with steam to separate most of the biphenyl formed during the reaction. As aucuparin methylether also slowly distils with steam, the distillation was not carried to completion. The oily residue was distilled under reduced pressure giving a colourless oil (0.5 g) which solidified on standing. This solid was chromatographed on silica gel (50 g). Benzene eluted in the following order: Biphenyl (0.05 g), a mixture of 3,4,5-trimethoxybiphenyl and 3,4,5,3',4',5'-hexamethoxybiphenyl (0.04 g), and pure hexamethoxybiphenyl (0.39 g), m.p. 123—124° (lit.[6] 126°). The separation of the tri- and hexamethoxybiphenyls was incomplete and repeated chromatography did not improve the separation. The mixture was therefore distilled in a gradient tube (0.1 mm Hg; temperature gradient from 150° to room temperature). The product from the colder part of the tube gave, after crystallisation from methanol, small amounts of *3,4,5-trimethoxybiphenyl* (1c), m.p. 84—89° undepressed on admixture with aucuparin methylether (I.R. spectra identical).

(b) Methyl 2-bromobenzoate (6.5 g) and 3,4,5-trimethoxyiodobenzene (2.9 g) were intimately mixed with copper bronze (25 g) and the mixture was kept at 240° for 10 min. The reaction mixture was allowed to cool and the organic products extracted with chloroform. After evaporation of the solvent the residue was dissolved in ether and filtered through alumina (neutral, Brockmann activity I, 2 g). The filtrate was evaporated to dryness and distilled with steam. The residue was saponified with ethanolic sodium hydroxide. Water was added and 3,4,5,3',4',5'-hexamethoxybiphenyl (0.7 g) removed by extraction with ether. The alkaline phase was acidified and extracted with ether. The ether extract was dried (Na_2SO_4) and the oily residue (1.8 g), after evaporation of the solvent, was chromatographed on silica gel (50 g). Ethanol (2 %) in chloroform eluted *2'-carboxy-3,4,5-trimethoxybiphenyl* (0.6 g), which after recrystallisation from benzene/light petroleum (b.p. 40—60°) had m.p. 136—137°. (Found: C 66.9; H 5.3; OCH_3 30.9. $C_{13}H_7O_2(OCH_3)_3$ requires C 66.7; H 5.6; OCH_3 31.3). Further elution with the same solvent mixture gave diphenic acid (0.7 g), m.p. 228—230°.

(c) Methyl 3-iodo-4-methoxybenzoate [7] (1.7 g), m.p. 95—97° (lit.[7] 94—95°), and 3,4,5-trimethoxyiodobenzene (3.4 g) were thoroughly mixed with copper bronze (11 g)

and the mixture was kept at 230° for about 10 min. The organic product was extracted with chloroform. The solvent was removed, the residue dissolved in ether and filtered through alumina (neutral; Brockmann activity I; 2 g). The resulting filtrate was evaporated to dryness, and the product saponified with ethanolic sodium hydroxide. Water was added and 3,4,5,3′,4′,5′-hexamethoxybiphenyl (1.2 g) removed by extraction with ether. The acids were then precipitated with dilute hydrochloric acid. The precipitate (1.6 g) was triturated with boiling methanol leaving the less soluble acid (0.9 g), 3,3′-dicarboxy-6,6′-dimethoxybiphenyl. (M.p. and mixed m.p. 308—311°. I.R. spectrum identical with that of an authentic sample [5]). The methanol soluble acid crystallised on cooling and gave, after recrystallisation from methanol, 5-carboxy-3,4,5,2′-tetramethoxybiphenyl (0.5 g), m.p. 216—217.5°. (Found: C 64.2; H 5.7; OCH_3 38.4. $C_{13}H_6O_2(OCH_3)_4$ requires C 64.1; H 5.7; OCH_3 39.0).

The decarboxylations of 2′-carboxy-3,4,5-trimethoxybiphenyl and 5′-carboxy-3,4,5,2′-tetramethoxybiphenyl were carried out according to the following procedure.

The acid (0.10 g) in quinoline (3 ml) was refluxed for 3 h in an atmosphere of nitrogen in the presence of a copper chromite catalyst (Adkins catalyst, 0.05 g). The reaction mixture was filtered and the catalyst washed with ether. The combined organic phases were thorougly washed with dilute hydrochloric acid, dilute sodium hydroxide, and water. The ether phase was dried (Na_2SO_4) and evaporated to yield oily crystals (about 0.06 g), which were purified by chromatography on silica gel (3 g). Benzene eluted the pure product.

2′-Carboxy-3,4,5-trimethoxybiphenyl thus gave *3,4,5-trimethoxybiphenyl* (1c). The m.p. and I.R. spectrum were identical with those of aucuparin methyl ether. Mixed m.p. undepressed.

5′-Carboxy-3,4,5,2′-tetramethoxybiphenyl similarly gave 3,4,5,2′-tetramethoxybiphenyl (1d). The m.p., and I.R. spectrum were identical with those of methoxyaucuparin methyl ether. Mixed m.p. undepressed.

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