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PHENOLIC ACIDS AND PHENOLIC GLYCOSIDES OF *GAULTHERIA* SPECIES

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Abstract—Twenty-two species of *Gaultheria* were examined for phenols and phenolic acids obtained by hydrolysis of ethanolic extracts. Most species yielded *p*-hydroxybenzoic, *o*-pyrocatechuic, protocatechuic, gentisic, vanillic, *p*-coumaric, caffeic and ferulic acids. Thirteen species contained derivatives of salicylic acid as well; the glycoside gaultherin was isolated from two of these. The three species of the section Amblyandra yielded catechol as the major phenol in hydrolyzates of ethanolic extracts. Catechol-β-D-glucopyranoside was isolated from one of these. The relationship of these phenolic compounds to one another is discussed.

INTRODUCTION

ALTHOUGH methyl salicylate has been detected in other plants such as members of the Betulaceae[1] or the Polygalaceae[2] it is perhaps best known from *Gaultheria* (Ericaceae), particularly *G. procumbens* or wintergreen. It occurs in *G. procumbens*, *G. cumingiana* and *G. pyroloides*[3, 4] as gaultherin, a glucose-xyloside of methyl salicylate. Since a number of related hydroxybenzoic acids occur in *G. procumbens*[5] we considered it of interest to discover whether substituted benzoic acids, especially salicylic acid, are characteristic of the genus. As a number of species was made available to us a survey of the simpler phenolic constituents was undertaken.

RESULTS AND DISCUSSION

The results of this survey are presented in Table 1. Due to the small amounts of material of certain species which were available, it was not possible to isolate phenolic glycosides from all species. Gaultherin was isolated and identified from *G. procumbens* and *G. hispidula*. There is no record in the literature of the latter species having been examined before.

The species listed in the Table have been arranged, as far as we can ascertain, in accordance with the taxonomic views of Airy-Shaw.[6] It may be observed that there is, to a certain degree, a correlation between the distribution of salicylic acid and the classification suggested by him. *G. punctata* Bl. not examined here, is reported to yield methyl salicylate.[7] It has been placed in the section Leucothoides. Species in this section are consistent therefore in containing salicylate derivatives. *G. leucocarpa* Bl. (Section Gymnobotrys) also yields oil of

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[2] L. N. PRISTA and A. C. ALVES, *Garcia Orta* **6**, 131 (1958).
[3] M. YASUE and T. SASAKI, *Chem. Zentr.* **1**, 2428 (1939).
[4] T. SASAKI and Y. WATANABE, *J. Pharm. Soc. Japan*, **76**, 892 (1956).
[5] R. K. IBRAHIM and G. H. N. TOWERS, *Arch. Biochem. Biophys.* **87**, 125 (1960).
[6] H. K. AIRY-SHAW, *Kew Bull.* **5**, 306 (1940).
[7] J. C. UMNEY, *Perfumery Essent. Oil Record* **5**, 69 (1914).

Table 1. Distribution of phenolic acids and catechol in hydrolyzates of ethanolic extracts of species of *Gaultheria*

Species	Phenolic compounds*											
	catechol	salicylic acid	*p*-hydroxybenzoic acid	*o*-pyrocatechuic acid	gentisic acid	protocatechuic acid	vanillic acid	syringic acid	*p*-coumaric acid	caffeic acid	ferulic acid	sinapic acid
Section 1. Brossaeopsis												
G. nummularioides D. Don	−	−	+	+	+	+	+	−	+	+	+	+
G. nummularioides D. Don var. elliptica Rehd. et Wils.	−	t	+	+	+	+	+	−	+	+	+	+
G. shallon Pursh.	−	−	+	+	+	+	+	+	+	++	+	+
Section 2. Amblyandra												
G. adenothrix (Miq.) Maxim.	++	−	−	+	+	+	+	−	−	+	−	−
G. ovatifolia A. Gray	++	−	−	+	+	+	−	−	−	+	−	−
G. humifusa (Grah.) Rydb.	++	−	−	+	+	+	+	−	−	+	−	−
Section 3. Leucothoides												
G. fragrantissima Wall.	−	++	+	+	+	+	+	−	+	+	+	−
G. cuneata (Rehd. et Wils.)	−	++	+	+	+	+	+	−	+	+	+	−
G. griffithiana Wight	−	++	+	+	+	+	++	−	+	+	+	+
G. hookeri C.B. Cl.†	−	++	−	+	+	+	++	−	+	+	−	−
G. itoana Hay	−	++	+	+	+	+	+	−	+	+	−	+
G. pyroloides Miq.‡	−	++	+	+	+	+	+	+	+	+	+	−
Section 4. Eugaultheria												
G. procumbens L.	−	++	+	++	++	+	++	+	+	++	+	−
G. depressa Hook f.	−	−	+	−	+	+	+	−	++	++	+	−
G. hispidula (L.) Muhlenh.	−	++	+	−	+	+	−	+	−	++	+	+
G. tetramera W.W. Sn.	−	+	+	+	+	+	+	−	+	+	+	+
G. thymifolia Stapf.	−	−	+	+	+	+	+	−	+	+	+	+
Section Unassigned												
G. yunnanense (Franck) Rehder.	−	++	−	+	+	+	+	−	+	+	+	−
G. rengifoana Rhil.	−	+	+	+	+	+	+	−	+	+	+	+
G. hispida R. Br.	−	−	+	−	+	+	+	−	+	+	+	−
G. wisleyensis§	−	−	+	+	+	+	+	−	+	+	+	+
G. eriophylla Mart.	−	−	−	+	+	+	+	+	+	+	+	+

* Major constituent is ++, minor constituent is + and t stands for trace amount.
† Received as *G. veitchiana*.
‡ Received as *G. Miqueliana*.
§ × *Gaulthettya wisleyensis* (Marchant) Rehder = *Gaultheria shallon* × *Pernettya mucronata* Gaudich. ex Don.

wintergreen[7] which brings the total number of species known to accumulate salicylate to 16 out of 25. As there are possibly 150 species of *Gaultheria* it would be of interest to know whether the distribution of salicylate is of any real taxonomic value.

It is of interest that the section Amblyandra, representing "a small isolated group" (Airy-Shaw), differs from all other species examined in having a derivative of catechol as the

major phenolic compound in ethanolic extracts. This group stands apart from all other species examined in the nature of its simpler phenolic compounds.

Chromatograms of crude ethanolic extracts of *G. ovatifolia* and *G. humifusa* indicated that free catechol was present in trace amounts only. A prominent phenolic compound on chromatograms was found to be replaced by catechol after emulsin treatment of extracts. Chromatographic data for this compound are given in Table 2.

TABLE 2. CHROMATOGRAPHIC DATA FOR CATECHOL-β-D-GLUCOPYRANOSIDE AND CATECHOL

Compound	R_f in solvents				Colour reactions	
	A	B	C	D	diazotized *p*-nitroaniline	Fast Bordeaux Red
Catechol	0·91	0·75	0·59	0·94	green-gray	blue fading to brown
Catechol glucoside	0·69	0·84	0·02	0·85	magenta	pink

Solvents: A—butanol–acetic acid–water (4:1:2·2); B—2% HCOOH; C—Upper phase benzene–acetic acid–water (10:7:3); D—pyridine–ethyl acetate–water (5:12:4).

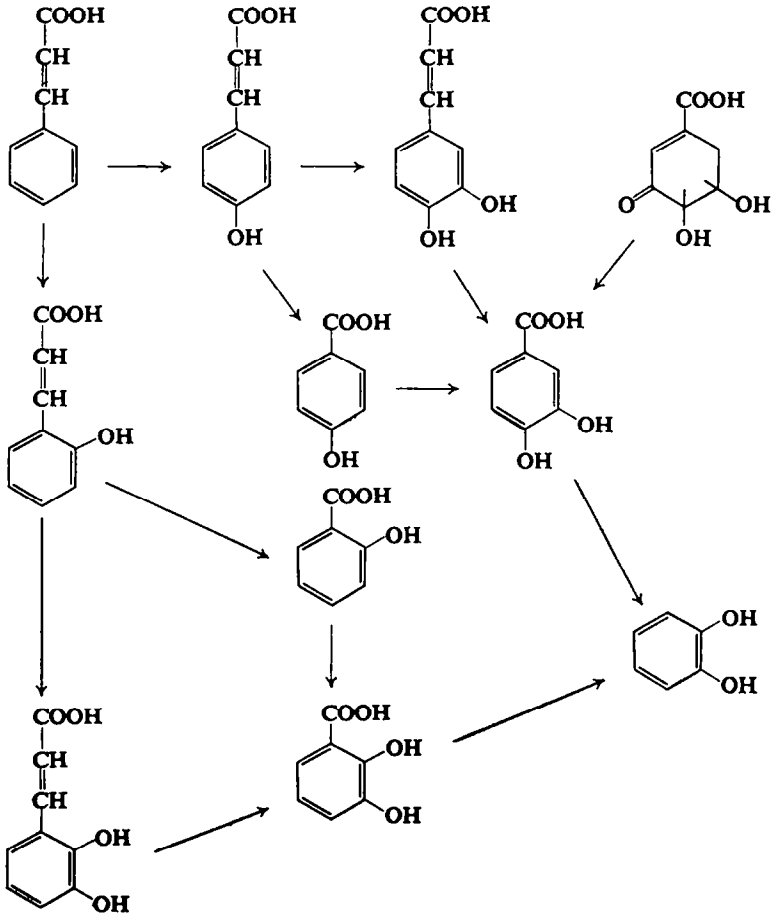

FIG. 1. POSSIBLE ROUTES TO THE BIOSYNTHESIS OF CATECHOL IN *Gaultheria*.

The compound was isolated from *G. ovatifolia* and identified as catechol-β-D-glucopyranoside. This is, as far as we know, the first report of the natural occurrence of this simple phenolic compound. Its obvious relationship to salicylic and *o*-pyrocatechuic acids on the one hand and to protocatechuic acid on the other indicate possible biogenetic origins of catechol (see Fig. 1).

EXPERIMENTAL

Identification of Phenolic Acids and Catechol

Ethanolic extracts of plant material (5–25 g) were subjected to emulsin or 2 N HCl hydrolysis followed by hydrolysis with 2 N NaOH in the cold. Hydrolyzates were extracted into ether and the concentrated ethereal extracts examined by two-directional paper chromatography using the method of Ibrahim and Towers.[5] Fast Bordeaux Red, a stabilized diazonium salt, diazotized *p*-nitroan-line or 1% $FeCl_3$ were used as spray reagents.

Isolation of Gaultherin from G. procumbens *and* G. hispidula

Fresh leaves (100 g) were extracted repeatedly with boiling ethanol until the extracts were colourless. The combined extracts were evaporated to dryness and the residue thus obtained was extracted with 150 ml boiling water and the mixture filtered through a bed of Celite. The cooled filtrate was passed on to a column (6 × 40 cm) of coconut charcoal which had been treated with N HCl and subsequently washed free of acid. The column was eluted successively with 1 l. each of water, 10% ethanol, 50% ethanol, absolute ethanol and 1:1 ethanol:benzene. The ethanol:benzene eluate was taken to dryness and the gummy residue dissolved in the minimum amount of hot absolute ethanol from which, on cooling, gaultherin crystallized. Two re-crystallizations from ethanol yielded 145 mg of colourless prisms, m.p. 181–183° uncorr. The yield from both species was about the same. (Found, C, 51·53; H, 5·63. Calc. for $C_{19}H_{26}O_{12}$, C, 51·11; H, 5·82%). Acid hydrolysis yielded glucose and xylose which were detected chromatographically.[8] Hydrolyses with emulsin followed by alkali yielded salicylic acid which was purified by sublimation. It was identical in its i.r. spectrum with the authentic compound and a mixed m.p. showed no depression.

The hexaacetate, prepared with acetic anhydride in pyridine, was re-crystallized from ethyl acetate:light petrol to give a compound melting at 190–191° uncorr. (Found, C, 52·92; H, 5·59. Calc. for $C_{31}H_{38}O_{18}$, C, 52·10; H, 5·32%).

Isolation of Catechol-β-D-glucopyranoside from G. ovatifolia

One kilogram of fresh leaves were extracted and chromatographed on coconut charcoal in the same way as for the isolation of gaultherin except that five times the amount of charcoal was used. In this case, however, the 50% ethanolic and the absolute ethanol extracts were found to contain the bulk of the glucoside. These ethanolic eluates were combined, taken to dryness on a rotary evaporator and the residue thus obtained extracted repeatedly with benzene to remove catechol which was present as a contaminant. All attempts to obtain a crystalline glucoside failed and led to partial hydrolysis. The material, freed of catechol again by benzene extraction, was dissolved in water and passed through a column (3 × 30 cm) of polyamide (Fluka & Co.). The glucoside fraction obtained was relatively free from catechol, but, however, did not yield a crystalline product. Remaining impurities were removed by continuous extraction (30 hr) of an aqueous solution of the glucoside with ethyl acetate. The

[8] L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.* 1702 (1950).

ethyl acetate extract yielded a cream coloured powder (2 g) which melted at 130–140°. Attempts to obtain a crystalline product were unsuccessful. The compound had λ_{max} 274mμ : ϵ_{max} 22,600, and $[\alpha]_D^{22} = -79 \pm 2°$ ($c = 0{\cdot}3\%$ in ethanol). Hydrolysis with emulsin yielded a stoichiometric amount of catechol which was isolated by benzene extraction and sublimation. Its i.r. spectrum was identical with that of authentic purified catechol and a mixed m.p. showed no depression. Glucose was identified as the other hydrolysis product by paper chromatography.[8]

The pentaacetate of catechol glucoside was prepared with acetic anhydride and pyridine. Five re-crystallizations from light petrol containing a trace of acetone yielded colourless needles m.p. 135·5–136·5 uncorr. (Found, C, 54·82; H, 5·41. Calc. for $C_{22}H_{26}O_{12}$, C, 54·77; H, 5·39%). $[\alpha]_D^{22} = -21 \pm 8°$ ($c = 0{\cdot}3\%$ in ethanol).

The NMR spectra of catechol glucoside and its penta-acetate were consistent with the assigned structures. The spectrum of the glucoside, obtained in pyridine, showed that the anomeric proton resonance at τ 5·65 was more complex than would have been expected on a "first-order" basis, due to virtual coupling between H_2 and H_3.[9] However, the half-height width of this resonance ($\sim$ 10 c/s) accords with the assigned β-configuration of this compound. The observation of the H_5 resonance of the pentaacetate, in deuterated chloroform, as a clearly separated multiplet to high field of the H_6 resonance also agrees with the expected β-configuration at C_1.[10]

Chromatographic evidence for the presence of catechol glucoside was obtained with extracts of *G. humifusa* and *G. adenothrix* as well as from berries of *G. ovatifolia.*

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[9] J. I. Mushes and G. J. Corey, *Tetrahedron*, **18**, 791 (1962).
[10] R. U. Lemieux and J. D. Stevens, *Can. J. Chem.* **43**, 2059 (1965).