# *Chemistry of the* Coprosma *Genus. Part VIII.* The Occurrence of Asperuloside.*

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Asperuloside has been shown by isolation or by colour reactions to occur in 73 *Coprosma* species. Additional occurrences in other species of the *Rubiaceae* are listed.

ASPERULOSIDE has been isolated in crystalline form from six members of the *Rubiaceae* family. On the basis of the test that on hydrolysis it gives first a green colour and then a black precipitate it has been detected in 14 other members of this family. It has been isolated from one member of the *Euphorbiaceae*. It is moreover probably identical with rubichloric acid (chlorigenin) which was obtained as a syrup from four other members of the *Rubiaceae*.

We have now isolated asperuloside from five *Coprosma* species (*Rubiaceae*), have detected its presence by colour reactions in 68 other species of this genus, and failed to detect in two others. We also record positive tests for 43 other plants, and negative tests for 57 others. Details of these and the earlier isolations are in the Experimental section.

The stability of asperuloside is indicated by the fact that the specimen of *C. solandri* Kirk collected by Banks and Solander during Captain Cook's visit to New Zealand in 1769 still gave a positive test.

Hérissey (*Compt. rend.*, 1925, **180**, 1695) described asperuloside as colourless, hydrated needles, m. p. 126—127°, $[\alpha]_D^{25}$ −195·5 (in $H_2O$), *M*, 410 (cryoscopic in water). No formula was suggested, but it was shown that a reducing sugar, presumed to be glucose, was produced on both acid and enzymic (emulsin) hydrolysis. Juillet, Susplugas, and Massa (*J. Pharm. Chim.*, 1938, **27**, 56) identified the sugar as glucose. The aglycone, asperuligenol, was an amorphous blackish-brown precipitate similar to that formed by the hydrolysis of aucubin (Bourquelot and Hérissey, *Ann. Chim. Phys.*, 1905, **4**, 289). Trim and Hill (*Biochem. J.*, 1951, **50**, 310) suggested a formula $C_{17}H_{24}O_{11}$ for asperuloside and prepared an acetyl derivative, m. p. 153°, regarded as the octa-acetate. Because of the similarity in colour reactions they suggested that asperuloside was a furan derivative similar to aucubin whose constitution has been elucidated by Karrer and Schmid (*Helv. Chim. Acta*, 1946, **29**, 525).

Asperuloside, as obtained by us, has m. p. 131—132°, after softening at 126°, the m. p. recorded by Hérissey. Combustions of the air-dried material indicate the formula $C_{18}H_{24}O_{12}$, which, from Hérissey's and our results, represent the hydrate, $C_{18}H_{22}O_{11},H_2O$. Since asperuloside is a glucoside it may be further represented as $C_{12}H_{11}O_5{\cdot}O{\cdot}C_6H_{11}O_5,H_2O$, the glucose being joined by a β-linkage since it is hydrolysed by emulsin. An acetate prepared by Nicholls (Theses, Univ. of New Zealand, 1946, 1948), m. p. 154°, is regarded as the tetra-acetate, $C_{18}H_{18}O_7(O{\cdot}CO{\cdot}CH_3)_4$. A tribenzoate has also been prepared. Degradative studies leading to the constitution will be reported later.

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## Experimental

Analyses are by Mr. R. N. Seelye of this Department and Dr. J. A. Mills, University of Adelaide.

*Plant Sources of Asperuloside.*—Asperuloside was first isolated in crystalline form by Hérissey (*Compt. rend.*, 1925, **180**, 1695; *J. Pharm. Chim.*, 1925, **2**, 177) from *Asperula odorata* (woodruff) and later from *Galium aparine* (*Compt. rend.*, 1926, **182**, 865; *Bull. Soc. Chim. biol.*, 1926, **8**, 489, 1208), *G. verum* (*Compt. rend.*, 1927, **184**, 1674), and *Coprosma repens* (syn. *C. baueriana*) (*J. Pharm. Chim.*, 1933, **17**, 553). Hérissey (*Bull. Soc. Chim. biol.*, 1926, **8**, 1208) considered that asperuloside is probably identical with rubichloric acid (chlorogenin), obtained only as a syrup by Rochleder (*Ann. Chim. Phys.*, 1851, **80**, 321) from *Rubia tinctorum* (madder), by Orth (*J. pr. Chem.*, 1855, **64**, 10) from *Gardenia grandiflora*, and by Perkin and Hummel (*J.*, 1893, **63**, 1160) from *Oldenlandia umbellata* (Indian madder) and *Morinda umbellata* (*J.*, 1894, **65**, 851). The identification was based on the characteristic reaction of asperuloside on acid hydrolysis, giving first a green colour and then a black precipitate. The same reaction was used by Hérissey (*Compt. rend.*, 1926, **182**, 865; 1927, **184**, 1674) to detect the presence of asperuloside in *Asperula tinctoria, Coprosma lucida, C. robusta, Crucianella stylosa, Galium cruciata, G. mollugo, Leptodermis lanceolata, Manettia bicolor, Paederia foetida, Putoria calabarica, Rubia peregrina, R. tinctorum, Serissa foetida*, and *Sherardia arvensis*. Juillet *et al.* (*loc. cit.*) isolated asperuloside from *Crucianella maritima* and *C. angustifolia*. All the above species are members of the *Rubiaceae*. More recently, however, Trim (*Nature*, 1951, **167**, 485; Trim and Hill, *loc. cit.*) isolated it from *Daphniphyllum macropodum*, a member of the *Euphorbiaceae*.

By acetone extraction we have isolated asperuloside from *Coprosma arborea* Kirk, *C. lucida* J. R. & G. Forst., *C. repens* Rich., *C. robusta* Raoul, and *C. tenuifolia* Cheesem., and through its characteristic colour reactions we have detected it in 68 other *Coprosma* species. These include all the species listed by Oliver (Bernice P. Bishop Museum Bull. 132, 1935, p. 202) with the exception of *C. baueri* Endl. and *C. benefica* Oliv., which gave negative tests, and *C. glabrata* Moore, *C. hookeri* (Don) Oliv., *C. moorei* Rod., *C. nivalis* Oliv., *C. novaehebridae* Oliv., *C. oceanica* Oliv., *C. persicaefolia* Gray, *C. pyrifolia* (Hook. & Arn.) Skotts., *C. raiateensis* Moore, *C. rapensis* Brown, *C. serrata* St. John, *C. setosa* Moore, *C. tadgelli* Oliv., *C. ternata* Oliv., and *C. wollastonii* Wern., which were not available.

Positive colour reactions characteristic of asperuloside were obtained from the following other members of the *Rubiaceae* : *Asperula perpusilla* Hook. f., *Diodia teres* Walt., *D. virginica* L., *Galium tenuicaule* A. Cunn., *Gardenia remyi* Mann, *Gouldia axillaris* Wawra, *G. purpurea* (Fosb.) Skottsb., *G. st.-johnii* Fosb., *G terminalis* (Hook. & Arn.) Hilleb. [including many varieties and forms described by Fosberg (Bernice P. Bishop Museum Bull. 147, 1937)], *Hedyotis acuminata* (Cham. & Schl.) Stend. f. *grayana* Fosb., *H. acuminata* (Cham. & Schl.) Stend. f. *sherffiana* Fosb., *H. angusta* Fosb. var. *umbrosa* Fosb., *H. centranthoides* (Hook. & Arn.) Stend., *H. elmeri* Merr., *H. fluviatilis* (Forbes) Fosb. var. *kamopuaana* (Degen) Fosb. f. *hathewayi* Fosb., *H. glaucifolia* (Gray) Fosb. var. *waimeae* (Wawra) Fosb., *H. schlechtendahliana* Stend. var. *cordata* (Cham. & Schl.) Fosb., *H. schlechtendahliana* Stend. var. *glabrescens* Fosb., *H. schlechtendahliana* Stend. f. *kaalensis* Fosb., *H. uniflora* DC., *H. vestita* R. Br., *Houstonia purpurea* L., *Kadua centranthoides* Hook. & Arn., *K. glomerata* Hook. & Arn., *K. grandis* A. Gray, *K. longipedunculata* Skottsb., *Mitchella repens* L., *Morinda bucidaefolia* A. Gray, *M. citrifolia* L., *M. forsteri* Seem., *M. rojoc* L., *M. trimera* Hillebr. var. *sanwicense* (Deg.) Skottsb., *M. umbellata* L., *Nertera cunninghamii* Hook. f., *N. depressa* Gaertn., *Normandia neo-caledonica* Hook. f., *Oldenlandia foetida* Forst. f., *Opercularia varia* Hook. f., *Oreopolus citrinus* Sch., *Paederia pringlei* Greenm., *Richardsonia scabra* A. St. Hil., *Spermacoce glabra* Michx., *Straussia hawaiiensis* A. Gray.

Negative tests were obtained from the following species which gave yellow, pink, red, reddish-brown colours or were without colour : *Asperula capitata* Kit., *A. ciliata* Roch., *A. aristata* L., *A. cynanchica* L., *A. galioides* DC., *A. taurina* L., *A. tenella* Boiss., *Bikkia grandiflora* Reinw., *Bobea elatior* Gand., *Bouvardia capitata* Bull., *Canthium odoratum* (Forst.) Seem., *Cephalanthus occidentalis* L., *Chiococca racemosa* Jacq., *Coffea arabica* L., *C. oxyloba* Janka, *Cruckshanksia hymenodon* Hook. & Arn., *Dentella repens* Forst., *Dolicholobium* sp., *Galium umbrosum* Forst., *Gardenia brighamii* Mann, *G. kaitensis* DC., *Guettarda ambigua* DC., *G. blodgettii* Shuttl., *G speciosa* L., *Hamelia patens* Jacq., *Hedyotis hispida* Retz., *H. microphylla* Merr., *H. schlechtendahliana* Stend. var. *rigida* Fosb., *Hintonia latiflora* (DC). Bull. var. *leiantha* Bull., *Ixora bracteata* Cheesem., *I. coccinea* L., *I. cumingiana* Vid., *Morinda bracteata* Roxb., *Mussaenda frondosa* L., *Nertera dichondrifolia* (A. Cunn.) Hook. f., *N. setulosa* Hook. f., *Ophiorrhiza leptantha* A. Gray, *Plectronia barbata* Benth. & Hook., *P. peduncularis* (Cav.) Vid., *Psychotria bullata* Seem., *P.*

*confertiloba* A. C. Sm., *P. forsteriana* A. Gray, *P. grandiflora* Mann, *P. hexandra* Mann, *P. hirtella* Skottsb., *P. insularum* A. Gray, *P. loniceroides* DC., *P. pinnatinervia* Elm., *Randia cumingiana* Vid., *R. graeffii* Rein., *Sarcocephalus cordatus* Miq., *Straussia hillebrandii* Rock, *S. kaduana* (Cham. & Schl.) A. Gray, *S. mariniana* (Cham. & Schl.) A. Gray, *S. psychotrichoides* Heller, *Timonius polygamus* (Forst.) Rob., and *Wendlandia luzoniensis* DC.

The stem bark of *Coprosma arborea* Kirk, *C. tenuifolia* Cheesem., *C. cuneata* Hook. f., *C. pumila* Hook. f., *C. repens* Rich., *C. rhamnoides* A. Cunn., and *C. robusta* Raoul is practically devoid of anthraquinone colouring matters but sucrose has been isolated from extracts of the two first-mentioned species.

*Isolation of Asperuloside.*—(a) *From* C. tenuifolia. The bark was obtained from a tree growing at Papa Aroha, near Coromandel. We are indebted to Dr. H. H. Allan for its identification. No colour reactions typical of anthraquinones were given by the bark. The air-dried, ground bark was extracted with acetone in a Soxhlet apparatus for 56 hr. During the extraction crystalline material separated which recrystallised from methyl alcohol (charcoal) as hexagonal prisms, m. p. and mixed m. p. with sucrose, 184°. The filtered extract, on concentration, deposited colourless needles of asperuloside (2·9%) which, after repeated crystallisation from ethyl alcohol, separated in needles, m. p. 131—132° after marked shrinking at 126°, $[\alpha]_D^{25}$ −198·6° (*l*, 1; c, 1·44 in $H_2O$) (Found, on air-dried material: C, 50·2, 50·1; H, 5·3, 5·5; OMe, 0; *C*-Me, 4·0, 3·95. $C_{18}H_{24}O_{12}$ requires C, 50·0; H, 5·6; 1*C*-Me, 3·5. Found, on material dried at 110° over $P_2O_5$: C, 52·15; H, 5·3. $C_{18}H_{22}O_{11}$ requires C, 52·2; H, 5·3%. Loss on drying: 3·7%. $C_{18}H_{22}O_{11},H_2O$ requires 4·17%). Asperuloside is not reduced at the dropping-mercury electrode.

(b) *From* C. arborea. The bark was collected from trees growing at Titirangi and Manurewa. The air-dried, ground bark, which gave no colour reactions typical of anthraquinones, was fractionally extracted with ethyl acetate (Soxhlet) for 100 hr. The first extraction (10 hr.). after standing for 2 days, was decanted from extremely viscous material. The latter was triturated with alcohol, washed with hot acetone, and crystallised from methyl alcohol, giving sucrose (0·8%), m. p. and mixed m. p. 180°, $[\alpha]_D^{25}$ +66·7° in $H_2O$ (Found: C, 42·2; H, 6·3. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·4%). The decanted material, together with the further extractions, on concentration deposited asperuloside (0·6%), m. p. 131—132°, after repeated crystallisation from acetone.

(c) *From* C. robusta *and* C. repens. Both barks from trees growing in the Auckland district were practically colourless, gave no colour reactions with sodium hydroxide solution, and after continuous extraction with acetone afforded asperuloside, m. p. 131—132°, in yields of 1·2 and 1·7% respectively.

(d) *From* C. lucida. The air-dried, ground bark was extracted with ether for 5 hr. and then with ethyl acetate for 60 hr. The ethyl acetate was concentrated in stages, the first material separating being the colouring matters described in Part IV (*J.*, 1949, 1241). The final concentrate crystallised when seeded with asperuloside, and afforded pure material, m. p. 131·5°, in the usual way.

All samples of asperuloside obtained above showed no depression of m. p. on admixture.

Asperuloside is soluble in water, alcohols (methyl to pentyl), acetone, ethyl acetate, dioxan, pyridine, and glacial acetic acid and insoluble in ether, benzene, chloroform, and ligroin. An aqueous solution is neutral but becomes yellow and finally reddish-brown on treatment with sodium hydroxide, carbonate, or hydrogen carbonate solution (more slowly with the weaker alkalis).

The formation of glucose on acid hydrolysis of asperuloside (Juillet *et al.*, *loc. cit.*) has been confirmed by the preparation of glucosazone, m. p. and mixed m. p. 203—204° (decomp.).

*Asperuloside Tetra-acetate.*—This derivative, prepared by the action of acetic anhydride in boiling pyridine, separated from alcohol in colourless plates, m. p. 154°, undepressed by a sample kindly supplied by Dr. A. R. Trim [Found, on material dried at room temperature: C, 53·3, 53·4, 53·6; H, 5·8, 5·8, 5·2; Ac, 43·3, 39·8. $C_{18}H_{18}O_7(O{\cdot}CO{\cdot}CH_3)_4$ requires C, 53·6; H, 5·2; 5Ac, 36·9%]. The anomalous acetyl values will be discussed later.

*Asperuloside Tribenzoate.*—Asperuloside (300 mg.), benzoyl chloride (1 c.c.), and pyridine (3 c.c.) were boiled under reflux for ½ hr., cooled, and poured on ice (100 c.c.). The viscous red oil which separated was triturated with alcohol and, after repeated crystallisation from the same solvent, separated in colourless needles, m. p. 165—166° [Found, after drying at 100°: C, 63·5; H, 5·1. $C_{18}H_{19}O_8(O{\cdot}CO{\cdot}C_6H_5)_3,\frac{1}{2}H_2O$ requires C, 63·6; H, 4·9%).

The test for asperuloside was carried out by heating a few fragments of the stem-bark for preference or, where necessary in the case of herbaceous material, the whole stem, with *ca.* 1 c.c. of 2N-hydrochloric acid. In most cases the very characteristic bluish-green colour appears on

boiling. In a few cases, there was doubtful coloration but, on standing, the presence of asperuloside was confirmed by the formation of the black precipitate of asperuligenol. With herbaceous material some negative tests may not necessarily be due to the absence of asperuloside but to the small scale on which the experiment was performed. Most of the material was obtained from the Cheeseman Herbarium, Auckland Institute and Museum, and we are greatly indebted to Dr. R. C. Cooper for assistance on the botanical aspects.

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