116. *Chemistry of the* Coprosma *Genus. Part I. The Colouring Matters from* Coprosma Australis.

By Lindsay H. Briggs and Jack C. Dacre.

Three colouring matters from the bark of *Coprosma australis* have been identified with the anthraquinone derivatives morindin, morindone, and rubiadin-1 methyl ether, the total yield of the three constituents reaching 17% of the dry weight. The same constituents have been identified in the root bark.

THE genus *Coprosma* with 90 species, one of the largest genera of the *Rubiaceæ* well known for their tinctorial properties, has a predominantly temperate distribution and is developed to its greatest extent in New Zealand where there exist 41 endemic species. The inner bark of numerous species has been used extensively by the Maoris for colouring flax fibre [Te Rangihiroa (Sir Peter Buck), " On the Maori Art of Weaving Cloaks, Capes and Kilts ", Dominion Museum Bulletin (New Zealand), 1911, No. 3]. During the Great War of 1914—1918 when khaki dyes were scarce, Aston (*New Zealand J. Sci. Tech.*, 1918, **1**, 3, 264, 346; *New Zealand J. Agric.*, 1917, **15**, 55, 117; 1918, **16**, 358; 1919, **17**, 136; 1923, **26**, 78) investigated numerous species and found that many had mordant dye properties. He also drew attention to the wide individuality shown by the cortical tissue and the colour produced by the action of dilute sodium hydroxide solution. The dyeing properties of this genus have also been elaborated by Hutchinson (" Plant Dyeing ", Telegraph Print, Napier, New Zealand, 1943) for the home dyer.

C. australis (syn., *C. grandifolia*) is a large shrub 8—15 feet high occurring abundantly from sea level to 2,000 feet throughout the North Island and the Marlborough and Nelson Provinces of the South Island of New Zealand. The bark is coloured orange on the inner surface, and is quite thick and readily stripped from the trunk. The sap from the inner bark has been used by the early Maoris as a cure for scabies (" hakihaki ") (Goldie, *Trans. Roy. Soc. New Zealand*, 1904, **37**, 1) but Skey (*ibid.*, 1869, **2**, 152) could not detect the presence of alkaloids. Aston, however (*New Zealand J. Agric.*, 1017, **15**, 117; 1918, **16**, 358; *New Zealand J. Sci. Tech.*, 1918, **1**, 3), isolated two coloured crystalline compounds *A* and *B* from the stem bark of this species in 0·068% yield. Compound *A* melted at 270—272° and sublimed in iridescent brick-red needles at *ca.* 200° with a violet vapour, while compound *B* melted at 285—286° and sublimed above 200° in canary-yellow crystals. The colour reactions were typical of anthraquinone derivatives but the compounds were not analysed or identified.

In a later investigation on the same plant, Denz (Jacob Joseph Scholarship Thesis, Victoria University College, New Zealand, 1933) separated three compounds, *A*, $C_{14}H_8O_3$, m. p. 302°, which he considered to be β-hydroxyanthraquinone since on fusion with potassium hydroxide it allegedly yielded alizarin, *B*, orange-yellow needles, m. p. 230°, and *C*, for which no details were given.

With the kind permission of Mr. B. C. Aston we are continuing work in this field. The material for this investigation was collected from Kauri Gully, near Auckland. By exhaustive extraction of the air-dried bark with alcohol or acetone a completely crystalline product was obtained, the total extract reaching 17% of the dry weight of bark. Through different solubilities in solvents three compounds *A*, *B*, and *C* have been isolated.

Compound *A*, $C_{27}H_{30}O_{14}$, m. p. 264·5° (decomp.), is, we consider, morindin, the water-soluble glycoside of morindone (1 : 5 : 6-trihydroxy-2-methylanthraquinone)—a constituent not isolated by Aston or Denz. The melting point is considerably higher than that previously recorded but varies considerably with the rate of heating. However, an authentic specimen had a similar melting point when taken at the same rate of heating (as the compound decomposes at its melting point any mixed melting point is without significance). A crystalline acetate and benzoate have been recorded for morindin but only amorphous derivatives were obtained in this case. The compound is a rhamnoglucoside of morindone since on hydrolysis with dilute acids it yields morindone and a mixture of sugars from which rhamnose was identified as its *p*-nitrophenylhydrazone and glucose as its osazone. The two sugars are probably present as a disaccharide attached to the β-hydroxy-group since methylation with excess of the reagent gave a product which still furnished morindone on hydrolysis (in the anthraquinone series α-hydroxyl groups are methylated with difficulty in contrast to β-hydroxyl groups which are readily methylated). All the earlier formulæ suggested for morindin do not agree with this rhamnoglucoside structure and should be abandoned. A curious property of this compound, hitherto unobserved, is that on boiling with water for a short time it is transformed into a water-insoluble isomeric form of almost identical melting point and rotation.

Compound *B*, $C_{15}H_{10}O_5$, m. p. 284·5°, has been identified as morindone by its melting point, colour reactions, and the preparation of the triacetate, tribenzoate, and mono- and tri-methyl ethers. A *dibenzoate* has also been prepared. This compound agrees with compound *A* of Aston. Morindone, in the free state or as its glycoside, morindin, also occurs naturally in *Morinda umbellata*, *M. citrifolia* and *M. tinctoria* (Klein, " Handbuch der Pflanzenanalyse ", 1932, Bd. III, p. 1034). The aglycone has been synthesised by Jacobsen and Adams (*J. Amer. Chem. Soc.*, 1925, **47**, 285) and by Bhattacharya and Simonsen (*J. Indian Inst. Sci.*, 1927, **10**A, 6).

Compound *C*, $C_{16}H_{12}O_4$, m. p. 302°, has been identified as rubiadin-1 methyl ether (3-hydroxy-1-methoxy-2-methylanthraquinone) by its melting point, colour reactions, and the

preparation of the dimethyl ether and acetate. A *benzoate* has also been prepared. This compound agrees with compound *B* of Aston and compound *A* of Denz despite the alleged conversion of the latter into alizarin. Rubiadin-1 methyl ether has already been found in the root bark of *citrifolia* (Simonsen, *J.*, **1920**, **117**, 561), *M. longiflora* (Barrowcliff and Tutin, *ibid.*, 1907, **91**, 1909), *C. areolata*, and *C. rubra* (forthcoming publications), its constitution following from its synthesis by Jones and Robertson (*ibid.*, **1930**, 1699).

Although the three compounds can be separated fairly readily by fractional crystallisation this is more conveniently done in other ways. Morindin is easily obtained in crystalline form by extracting the air-dried bark with hot alcohol. Morindone and rubiadin-1 methyl ether are best obtained by acetone extraction of the air-dried bark from which the water-soluble morindin has first been removed by water soaking. Separation is then effected by taking advantage of the fact that by addition of calcium or barium hydroxide solution, morindone forms an insoluble purple salt and rubiadin-1 methyl ether a red soluble salt from which the free compounds are liberated on acidification.

In order to detect the number of coloured compounds in the bark an acetone solution was chromatographed on magnesium oxide and developed with the same solvent. Three distinct bands were formed, a top purple lake which could not be developed, a middle pinkish-red layer readily developed, and a bottom light-orange band which could be eluted with ease. On addition of dilute hydrochloric acid to the top band a red precipitate formed, identified after crystallisation as morindone. The bottom layer after complete elution and removal of the solvent yielded pure rubiadin-1 methyl ether, while extraction of the middle band with dioxan furnished morindin. No other bands could be detected. By chromatographing an acetone extract of the air-dried root bark the same three compounds were identified by a similar procedure.

The three constituents are present in the approximate proportion of morindin 40%, morindone and rubiadin-1 methyl ether each 30%. The presence of morindone and rubiadin-1 methyl ether together in the same plant contradicts the second empirical rule of Mitter and Biswas (*J. Indian Chem. Soc.*, 1928, **5**, 769) relating to the constitution of naturally occurring anthraquinone derivatives.

Morindone and morindin (after hydrolysis) are mordant dyes (cf. Oesterle and Tisza, *Arch. Pharm.*, 1907, **245**, 534; Perkin and Hummel, *loc. cit.*), and the phenomenonally high yield of dyes (cf. madder which contains 4% in the root bark) makes this tree extremely valuable to the home-dyer. Our dyeing results on wool, however, are somewhat different from those of Perkin and Hummel who used a partly purified extract of the root-bark of *M. umbellata*, containing morindone as the chief dye, and those of Oesterle and Tisza who used pure morindone on cotton-wool. Our experiments have led to rather lighter tones and are recorded in detail in the experimental section.

This is the first of a series of papers in which we hope to make a comprehensive survey of the *Coprosma* genus. The results may then also be of value in contributing to the problem of hybridisation which frequently occurs among members of this genus (cf. Allan, *Aust. New Zealand Ass. Adv. Sci.*, 1930, **20**, 429).

Experimental.

The cleaned, air-dried, finely broken bark was extracted exhaustively with alcohol in a Soxhlet. On cooling, orange-yellow needles (*A*) separated, followed after concentration by orange-red needles (*B*) and finally bright yellow needles (*C*). Alternatively, after removal of *A*, the mother liquors were taken to dryness under reduced pressure, and excess of calcium or barium hydroxide was added to the crystalline residue and warmed. Compound *C* forms a red soluble salt and *B* a purple insoluble salt with both reagents. The insoluble salt was collected and washed until the filtrate was colourless. The combined red filtrate was acidified, liberating *C* as yellow flocks, while *B* was liberated as orange-red flocks on treating a suspension of the insoluble salt with dilute hydrochloric acid. All three compounds were readily crystallised from glacial acetic acid.

Compounds *B* and *C* were more conveniently obtained by extracting water-soaked bark with acetone, distilling off the solvent, and working up with lime water as above.

Compound *A* is apparently identical with morindin. It forms long bright orange-yellow needles from glacial acetic acid, m. p. 264·5° (decomp.), $[\alpha]_D^{25°}$ — 90·9° (*l*, 1; *c*, 0·054 in dioxan) [Found (on air-dried material): C, 53·5, 53·4; H, 5·6, 5·7; loss on drying at 115°/vac., 3·82. Calc. for $C_{27}H_{30}O_{14}$, $1\frac{1}{2}$ H_2O: C, 53·55; H, 5·5; $1\frac{1}{2}$ H_2O, 4·5%]. The compound readily dissolves in cold water but, on boiling, a water-insoluble form, *β-morindin*, separated in long orange-yellow needles, m. p. 264° (decomp.), $[\alpha]_D^{25°}$ — 92·4° (*l*, 1; *c*, 0·055 in dioxan) (Found: C, 55·9, 56·4, 56·15; H, 5·3, 5·4, 5·4. $C_{27}H_{30}O_{14}$ requires C, 56·1; H, 5·2%). Both isomerides are completely soluble in dioxan, pyridine, acetone, and methyl alcohol, slightly soluble in ethyl alcohol and glacial acetic acid, and insoluble in ether, chloroform, benzene, and light petroleum. They give identical pinkish-red solutions with 10% sodium hydroxide solution, dilute ammonia, and concentrated sulphuric acid, the last solution changing to purple on standing, indicating hydrolysis to free morindone.

Hydrolysis of Morindin.—Morindin (735 mg.) was hydrolysed by heating with 2% sulphuric acid (75 c.c.) at 100° for 8 hours. After cooling, the amorphous precipitate crystallised from glacial acetic acid in needles, m. p. 284°, undepressed by pure morindone.

The filtrate from the hydrolysis was warmed and shaken with barium carbonate and the neutral liquid concentrated to a few c.c. During some weeks this deposited very large crystals, m. p. 87—88°, characteristic of rhamnose. The *p*-nitrophenylhydrazone, prepared from 100 mg. of the crystalline material, after recrystallisation from alcohol had m. p. 191·5° undepressed by an authentic specimen of rhamnose *p*-nitrophenylhydrazone.

A portion of the original sugar solution was treated with phenylhydrazine in the normal way. The crystalline product was repeatedly recrystallised from 60% alcohol, then dissolved in boiling acetone, and the solution concentrated to half volume and allowed to cool. The product after similar treatment with acetone had m. p. 204°, undepressed by authentic glucosazone.

Methylation of Morindin.—Morindin (105 mg.) dissolved in dry acetone (70 c.c.), anhydrous potassium carbonate (2 g.), and methyl sulphate (0·5 c.c.) were heated under reflux for 4 hours. The same quantities of potassium carbonate and methyl sulphate were again added and heating continued for another 4 hours. The red insoluble potassium salt was separated from the acetone layer, dissolved in water and hydrolysed with dilute sulphuric acid. Morindone was thus obtained (yield 15 mg.), m. p. 282° after crystallisation, undepressed by a pure specimen.

Compound *B* crystallises from glacial acetic acid in orange-red needles with a bronze lustre, m. p. 284·5°, and has been identified with morindone, for which Jacobsen and Adams (*loc. cit.*) record m. p. 281—282° but other workers somewhat lower values [Found: C, 66·6; H, 4·0; *M*(micro-Rast), 305. Calc. for $C_{15}H_{10}O_5$: C, 66·7; H, 3·7%; *M*, 270]. It is readily soluble in pyridine and acetone, slightly soluble in dioxan, ether, glacial acetic acid, and methyl and ethyl alcohol, and insoluble in water, benzene, light petroleum, and chloroform. It gives violet solutions with concentrated sulphuric acid and 10% sodium hydroxide solution.

The acetate, formed by heating morindone (30 mg.) with acetic anhydride (5 c.c.) and anhydrous sodium acetate (1·5 g.) for 2 hours, was repeatedly crystallised from glacial acetic acid and formed pale yellow needles, m. p. 259° (yield 25 mg., 57%). Jacobsen and Adams (*loc. cit.*) record m. p. 255—256·5° and other workers considerably lower values.

Both the mono- and the tri-methyl ether were obtained on methylation. A solution of morindone (134 mg.) in dry acetone (40 c.c.) and methyl sulphate (0·5 c.c.) was heated with dry potassium carbonate (2 g.) for 30 minutes. The dark-red insoluble material was filtered off and acidified with dilute hydrochloric acid. The yellow precipitate crystallised from glacial acetic acid (yield 50 mg., 40%) in orange-red needles, m. p. 256·5°, unchanged by further recrystallisation. Simonsen (*loc. cit.*) records m. p. 248° for the monomethyl ether. The yellow filtrate, after similar treatment with half the above reagents, was then poured into water (100 c.c.). The dark-yellow oil formed crystallised from glacial acetic acid in characteristic fox-tail-like aggregates, m. p. 226° (yield, 27 mg., 16%). Simonsen (*loc. cit.*) gives m. p. 229° for the trimethyl ether.

The tribenzoate was formed by allowing a mixture of morindone (100 mg.), pyridine (4 c.c.), and benzoyl chloride (2 c.c.) to stand overnight. After being warmed for 10 minutes the solution was poured into water, the viscid oil produced solidifying on trituration with alcohol. After crystallisation from glacial acetic acid it formed light-yellow plates, m. p. 234° (yield 140 mg., 70%). Jacobsen and Adams (*loc. cit.*) record m. p. 233—234° and Simonsen (*loc. cit.*) m. p. 218—219°, for the tribenzoate.

A *dibenzoate* was prepared by warming a solution of morindone (108 mg.) in pyridine (3 c.c.) and benzoyl chloride (1 c.c.) for a few minutes, and pouring into water (200 c.c.). The coloured oil formed was washed with 5% sodium carbonate solution and then crystallised from glacial acetic acid to form bright orange-yellow prisms, m. p. 206·5°, unchanged by further recrystallisation (yield 131 mg., 68%) (Found: C, 72·85, 73·0; H, 3·9, 4·2. $C_{29}H_{18}O_7$ requires C, 72·8; H, 3·8%). This is probably the 2 : 5-derivative.

Compound *C*, bright-yellow needles or plates from glacial acetic acid, m. p. 302°, has been identified with rubiadin-1 methyl ether [Found: C, 71·7, 71·5, 71·6; H, 4·8, 4·7, 4·3; *M*(micro-Rast), 240. Calc. for $C_{16}H_{12}O_4$: C, 71·6; H, 4·5%; *M*, 268]. The m. p. is *ca.* 10° higher than that recorded by other workers [cf. Barrowcliff and Tutin (*loc. cit.*), Simonsen (*loc. cit.*), and Jones and Robertson (*loc. cit.*)]. Rubiadin-1 methyl ether is freely soluble in acetone, pyridine, and dioxan, slightly soluble in ether, glacial acetic acid, toluene, and methyl and ethyl alcohol, and insoluble in water, benzene, chloroform, and light petroleum. It gives orange-red solutions with concentrated sulphuric acid and sodium hydroxide solution.

The acetate, prepared by heating rubiadin-1 methyl ether (90 mg.) with acetic anhydride (1 c.c.) and anhydrous sodium acetate (100 mg.) for 2 hours and pouring into water (200 c.c.), crystallised from ethyl acetate in long lemon-yellow prisms, m. p. 183° (yield 90 mg., 75%) unchanged by further recrystallisation. Barrowcliff and Tutin (*loc. cit.*) record m. p. 173°, and Simonsen (*loc. cit.*) and Jones and Robertson (*loc. cit.*) m. p. 174°.

Rubiadin dimethyl ether was prepared by heating a solution of rubiadin-1 methyl ether (90 mg.) in dry acetone (40 c.c.) and methyl sulphate (1 c.c.) with dry potassium carbonate (2 g.) for ½ hour. The acetone layer was then poured into water (50 c.c.); the crystalline product (yield 80 mg., 86%) recrystallised from alcohol in pale yellow needles, m. p. 161° (Found: C, 72·7; H, 5·3. Calc. for $C_{17}H_{14}O_4$: C, 72·3; H, 4·9%). Barrowcliff and Tutin (*loc. cit.*) reported m. p. 181° for this derivative, a result queried by Jones and Robertson (*loc. cit.*) who obtained m. p. 158°.

The *benzoate* was obtained by heating a solution of rubiadin-1 methyl ether (197 mg.) in pyridine (5 c.c.) and benzoyl chloride (1 c.c.) for a few minutes and then pouring into water (200 c.c.). The dark yellow oil produced after treatment with 5% sodium carbonate solution crystallised from glacial acetic acid in yellow orthorhombic prisms (yield 120 mg., 45%), m. p. 160° (Found: C, 74·0; H, 4·3. $C_{23}H_{16}O_5$ requires C, 74·2; H, 4·3%).

Chromatographic Separation.—Preliminary experiments with a number of adsorbents showed that

magnesium oxide was the most suitable. A clear acetone extract was added to a series of columns (8 ins. × ½ in.) of this absorbent and developed with the same solvent. The top purple band was washed with acetone until a clear band separated it from the next. The purple lake was then suspended in water and acidified with dilute hydrochloric acid. The red flocculent precipitate was collected, well washed with water, and crystallised from glacial acetic acid to form orange-red needles, m. p. 284°, undepressed by pure morindone. The bottom light-pink band was eluted completely with acetone; the yellow solution on concentration yielded bright yellow needles, m. p. 302°, undepressed by pure rubiadin-**1** methyl ether. The central light-orange band was extruded and extracted with boiling dioxan. After concentration of the extract under reduced pressure water-soluble orange needles separated, m. p. 260° (decomp.), identified with morindin by its m. p. and colour reactions.

Dyeing Properties of Morindone.—The dye was applied in aqueous solution containing 25% acetone–alcohol to mordanted wool with the following results. Iron (ferric), brownish-black; copper, purplish; potassium dichromate, aluminium, reddish purple; uranium, very dark green; lead, nickel, lilac tones; tin, bright-red; molybdenum, titanium, bismuth, reddish tones; zinc, zirconium, light chocolate; cadmium, light brown; manganese, fawn.

The analyses are by J. Mills, Adelaide University, Drs. Weiler and Strauss, Oxford, and R. N. Seelye of this Department.

We are indebted to the Chemical Society, the Royal Society of New Zealand, and the Department of Scientific and Industrial Research, New Zealand, for grants, to Imperial Chemical Industries Ltd. for gifts of chemicals, and to Dr. Cross for anthraquinone derivatives and an authentic specimen of morindin from the collection of colouring matters of the late Professor A. G. Perkin.

AUCKLAND UNIVERSITY COLLEGE, NEW ZEALAND. [*Received, May* 14*th*, 1947.]