The Minor Anthraquinones of *Xanthoria parietina* (L.) Beltram, the Chlorination of Parietin, and the Synthesis of Fragilin and 7-Chloro-emodin ('AO-1')

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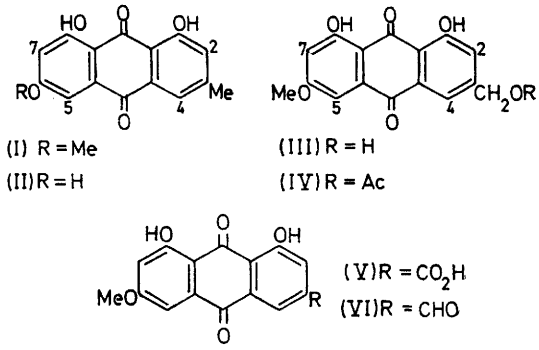
Extraction of the lichen *X. parietina* has yielded the anthraquinones emodin (II), teloschistin (III), and parietinic acid (V), as well as parietin (I). Chlorination of parietin (I) with chlorine (1 mol.) gave 5-chloroparietin (XVI), with chlorine (2 mol.) gave chiefly 4,5-dichloroparietin (XVII), and with an excess of chlorine gave 4,5,7-trichloroparietin (XVIII). Dechlorination of 4,5,7-trichloroparietin (XVIII) with an excess of sodium dithionite gave parietin (I), and with a deficiency of sodium dithionite gave, after methylation, tri-*O*-methylemodin and 4,5,7-trichlorodi-*O*-methylparietin. Dechlorination of 4,5,7-trichloroparietin (XVIII) with hydrazine hydrate and palladium–charcoal, after methylation, furnished di-*O*-methylfragilin (XIX). Partial demethylation of di-*O*-methylfragilin (XIX) yielded fragilin (IX), and complete demethylation of the former compound afforded 7-chloro-emodin (' AO-1 ') (VIII).

We required quantities of the anthraquinone parietin (I) which occurs commonly in various lichens.[1] Consequently we extracted the orange wall lichen *Xanthoria parietina* (L.) Beltram, collected in Kent and in New South Wales, which is a known source of parietin (I).[1] Most of the parietin (I) was obtained by direct crystallisation of the crude extract; examination of the mother liquors by t.l.c. revealed the presence of three other orange pigments. These were separated by preparative layer chromatography and on the basis of chemical and spectroscopic evidence (see Experimental section) were proved to be the known anthraquinones emodin [1] (II), teloschistin (fallacinol) [2-4] (III), and parietinic acid [5] (V). One method of isolation inadvertantly afforded teloschistin (III) as the artifact mono-*O*-acetylteloschistin (IV), but a milder method of isolation gave teloschistin (III) itself. The initial crude ex-extract was shown to contain teloschistin (III) and not the acetate (IV), by t.l.c. Teloschistin has previously been obtained from the lichens *Teloschistes flavicans* Norm.,[2] and *Xanthoria fallax* (Hepp.) Arn.[4] In the latter case it co-occurs with the aldehyde fallacinal (VI). Parietinic acid (V) has previously been isolated from *X. parietina*.[4]

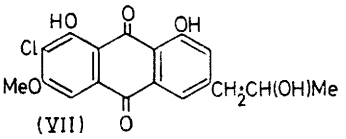
The number of known naturally occurring chlorine-containing anthraquinones has greatly increased recently. Most of these compounds have an arrangement

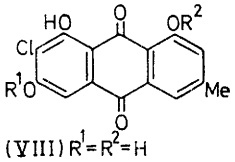


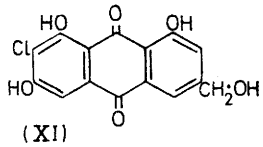
of substituents similar to that in emodin (II). The first known compound of this type was the mould metabolite nalgiolaxin [6,7] (VII). More recently the emodin-type compounds 7-chloro-emodin [8-11] (' AO-1 ') (VIII), fragilin [10,12] (IX), 7-chloro-1,6-di-*O*-methylemodin [10] (X),

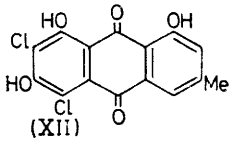
[1] R. H. Thompson, ' Naturally Occurring Quinones,' Butterworths, London, 1957.
[2] T. R. Seshadri and S. S. Subramanian, *Proc. Indian Acad. Sci.*, 1949, **30***A*, 67; S. Neelakantan, S. Rangaswami, T. R. Seshadri, and S. S. Subramanian, *ibid.*, 1951, **33***A*, 142.
[3] S. Neelakantan and T. R. Seshadri, *J. Sci. Ind. Res., India*, 1954, **13***B*, 884; S. Neelakantan, T. R. Seshadri, and S. S. Subramanian, *Proc. Indian Acad. Sci.*, 1956, **44***A*, 42.
[4] T. Murakami, *Chem. and Pharm. Bull.* (*Japan*), 1956, **4**, 298.
[5] W. Eschrich, *Biochem. Z.*, 1958, **330**, 73.
[6] H. Raistrick and J. Ziffer, *Biochem. J.*, 1951, **49**, 563.
[7] A. J. Birch and K. S. J. Stapleford, *J. Chem. Soc.* (*C*), 1967, 2570.
[8] Y. Yamamoto, N. Kirayama, and S. Arahata, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 304.
[9] I. Yosioka, H. Yamauchi, K. Morinoto, and I. Kitigawa, *Tetrahedron Letters*, 1968, 1149, 3749.
[10] G. Bendz, G. Bohmann, and J. Santesson, *Acta Chem. Scand.*, 1967, **21**, 2889.
[11] C. H. Fox, W. S. G. Maass, and T. P. Forrest, *Tetrahedron Letters*, 1969, 919.
[12] T. Bruun, D. P. Hollis, and R. Ryhage, *Acta Chem. Scand.*, 1965, **19**, 839.

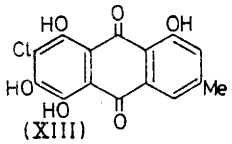
2-chloro-1,3,8-trihydroxy-6-hydroxymethylanthraquinone [8] (XI), 5,7-dichloro-emodin [9] ('AO-2', XII), have been isolated from lichens or moulds. Papulosin (XIII) is a 7-chloro-emodin containing a further nuclear hydroxy-group which has been isolated from a lichen.[11]


(VII)

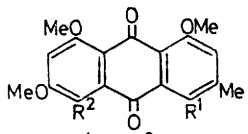

(VIII) $R^1=R^2=H$
(IX) $R^1=Me, R^2=H$
(X) $R^1=R^2=Me$

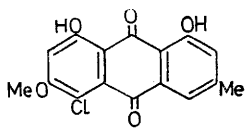

(XI)


(XII)

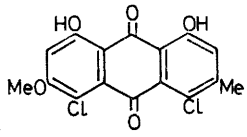

(XIII)

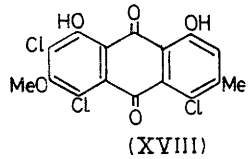
We have consequently examined the chlorination of parietin (I) as a possible route to some of these compounds which does not involve ring synthesis. Parietin (I) has previously been obtained by partial methylation [13,14] of emodin (II), which has also been synthesised.[15] The bromination of emodin (II), first examined in 1888, was reported to give both a mono- and a dibromo-compound, but no structural assignments were made.[16] More recently the mono-bromination of tri-*O*-methylemodin was reported to give 5-bromo-tri-*O*-methylemodin (XIV). This structural assignment was supported by replacement of the bromo-substituent with a methoxy-group, followed by partial demethylation of the product to afford 1,4,5-trihydroxy-2-methoxy-7-methylanthraquinone, the m.p. of which differed from that of erythroglaucin (1,4,5-trihydroxy-7-methoxy-2-methylanthraquinone).[17] In contrast to these results Brockmann and Kluge [18] have claimed that bromination of tri-*O*-methylemodin by an undisclosed method afforded 4-bromotri-*O*-methylemodin (XV). We have found that treatment of parietin (I) with chlorine (1 mol.) in chloroform at room tem-

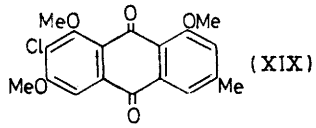

(XIV) $R^1=H, R^2=Br$
(XV) $R^1=Br, R^2=H$


(XVI)

perature gave 5-chloroparietin (XVI) in 88% yield. Chlorination of parietin under the same conditions but with chlorine (2 mol.) gave both 5-chloroparietin (XVI) and 4,5-dichloroparietin (XVII), which were separated by preparative layer chromatography. On chlorination of parietin with an excess of chlorine in acetic acid 4,5,7-trichloroparietin (XVIII) was obtained. These structural assignments follow unambiguously from the n.m.r. spectra of the chloroparietins and of their di-*O*-methyl ethers (see Experimental section).


(XVII)


(XVIII)


(XIX)

In the anthraquinone series α-halogeno-substituents may be removed selectively in the presence of β-halogeno-substituents.[19] Thus it was anticipated that 4,5,7-trichloroparietin (XVIII) could be converted into fragilin (IX). Indeed Yosioka and his co-workers [9] have reported that reduction of 5,7-dichloro-emodin ('AO-2') (XII) with dithionite in aqueous sodium hydroxide at room temperature, followed by aerial oxidation, gave 7-chloro-emodin ('AO-1') (VIII) and emodin (II). Treatment of 4,5,7-trichloroparietin (XVIII) with an excess of dithionite under similar conditions afforded parietin (I). Treatment of compound (XVIII) with a deficiency of dithionite gave after methylation and chromatography only tri-*O*-methylemodin and 4,5,7-trichlorodi-*O*-methylparietin. However, on boiling the trichloro-compound (XVIII) with 100% hydrazine hydrate (*ca.* 4·5 mol.) and 10% palladium–charcoal in ethanol, followed by methylation of the crude product and preparative layer chromatography, a 10·6% yield of di-*O*-methylfragilin (XIX) was obtained. Methylation of the crude product mixture from the dechlorination reaction was resorted to, since fragilin (IX) is only poorly separated from the other chloroparietins on t.l.c., whereas the methyl ethers may be separated more easily. Synthetic di-*O*-methylfragilin (XIX) was identical with a sample prepared by methylation of authentic fragilin.

Partial demethylation of di-*O*-methylfragilin (XIX) with hydrogen bromide gave synthetic fragilin (IX), which was identical with an authentic sample. Di-*O*-methylfragilin (XIX) was completely demethylated by the pyridine hydrochloride method, and gave 7-chloro-emodin ('AO-1') (VIII), identical with an authentic sample. Since 7-chloro-emodin ('AO-1')

[13] H. A. D. Jowett and C. E. Potter, *J. Chem. Soc.*, 1903, 1330.
[14] R. Eder and F. Hauser, *Helv. Chim. Acta*, 1925, **8**, 126, 140.
[15] See R. Eder and C. Widmer, *Helv. Chim. Acta*, 1923, **6**, 96; R. A. Jacobson and R. Adams, *J. Amer. Chem. Soc.*, 1924, **46**, 1312.
[16] P. Schwabe, *Arch. Pharm.*, 1888, **226**, 580; Y. Asahina and F. Fuzikawa, *Ber.*, 1935, **68**, 1558.
[17] O. Tanaka and C. Kaneko, *Chem. and Pharm. Bull.* (*Japan*), 1955, **3**, 284.
[18] H. Brockmann and F. Kluge, U.S.P. 2,707,704.
[19] See A. Kirchner, *Annalen*, 1887, **238**, 344; W. Junghans, *ibid.*, 1913, **399**, 316; F. Ullmann and O. Eiser, *Ber.*, 1916, **49**, 2166; F. Bayer, G.P. 236,604; B.A.S.F., G.P. 261,720.

(VIII) has previously been converted into 7-chloro-1,6-di-*O*-methylemodin [10] (X), the synthesis of 'AO-1' constitutes a formal synthesis of compound (X).

EXPERIMENTAL

General procedures have been given previously.[20] All methylations of hydroxy-quinones were carried out by the methyl sulphate–acetone–potassium carbonate method.

Extraction of X. parietina.—Samples of the lichen were collected in the Isle of Thanet region of Kent from walls, or between Narooma and Batemans Bay (Southern New South Wales) from rocks and cliff faces above the high tide level. The thallus was air-dried (35°) and powdered before extraction.

(*a*) The thallus (2·686 kg.) was extracted (Soxhlet) with acetone (20 l.). The acetone was removed at the pump and the residue crystallised from glacial acetic acid to afford parietin (16·41 g.). The mother liquors were evaporated to small volume, pre-adsorbed on to silica gel, and chromatographed over a column of the same material (total 1 kg.) with gradient elution by increasing concentrations of ethyl acetate in benzene. Early fractions afforded more parietin (3·10 g.); intermediate fractions contained three compounds as judged by t.l.c.; the tail fractions furnished parietinic acid which gave (from glacial acetic acid) fine orange needles (0·053 g.), m.p. and mixed m.p. with a synthetic sample [6] 301—303° (decomp.) * (lit.,[6] 304—305°); the i.r. spectra (KCl) of the two samples were identical. Methylation gave the *di-O-methyl ether methyl ester* which formed yellow needles from chloroform–ethyl acetate, m.p. 262—263° (Found: C, 63·8; H, 4·75. $C_{19}H_{16}O_7$ requires C, 64·05; H, 4·55%), τ 1·47 (1H, d, *J* 1·5 Hz, 4-H), 1·98 (1H, d, *J* 1·5 Hz, 2-H), 2·49 (1H, d, *J* 2·5 Hz, 5-H), 3·15 (1H, d, *J* 2·5 Hz, 7-H), 5·92 (3H, s, ester Me), and 5·99br (9H, s, 3 × OMe). Hydrolysis of the latter ester with ethanolic potassium hydroxide gave tri-*O*-methylemodic acid which formed yellow prisms, m.p. 267—270° (lit.,[14] 270°), from glacial acetic acid. The intermediate fractions from the column were reduced in volume and applied to several layer plates which were developed with 10% ethyl acetate–benzene. The fastest moving band was separated and extracted with chloroform, and yielded more parietin (total 19·60 g.). Parietin crystallised from ethyl acetate–light petroleum (b.p. 40—60°) as orange needles, m.p. 209—210° (lit.,[6] 206—207°) (Found: *M*, 284. Calc. for $C_{16}H_{12}O_5$: *M*, 284), τ −2·46 (1H, s, OH), −2·26 (1H, s, OH), 2·26br (1H, s, 4-H), 2·54 (1H, d, *J* 2·5 Hz, 5-H), 2·83br (1H, s, 2-H), 3·32 (1H, d, *J* 2·5 Hz, 7-H), 6·01 (3H, s, OMe), and 7·52 (3H, s, Me). The di-*O*-methyl ether formed fine yellow needles, m.p. 224—226° (lit.,[21] 224°), from ethyl acetate–benzene (Found: *M*, 312. Calc. for $C_{18}H_{16}O_5$: *M*, 312), τ 2·24br (1H, s, 4-H), 2·56 (1H, d, *J* 2·5 Hz, 5-H), 2·81br (1H, s, 2-H), 3·16 (1H, d, *J* 2·5 Hz, 7-H), 5·99 and 6·01 (total 9H, s, 3 × OMe), and 7·52 (3H, s, Me). The di-*O*-acetyl derivative formed yellow needles from ethanol, m.p. 185—186° (lit.,[6] 185—187°), τ 1·91br (1H, s, 4-H), 2·23 (1H, d, *J* 2·5 Hz, 5-H), 2·71br (1H, s, 2-H), 3·04 (1H, d, *J* 2·5 Hz, 7-H), 6·01 (3H, s, OMe), 7·50 (3H, s, Me), and 7·56 (6H, s, 2 × OAc). The middle band was separated and extracted with chloroform, and afforded *mono*-O-*acetylteloschistin* (0·045 g.) as orange needles, m.p. 193—195° (from methanol–chloroform) (Found: C, 63·3; 63·0; H, 3·55, 3·60. $C_{18}H_{14}O_7$ requires C, 63·15; H, 4·1%), $\nu_{max.}$ (KCl) 1740 (aliphatic acetate) cm.$^{-1}$; τ (CD_2Cl_2) −2·63 (1H, s, OH), −2·57 (1H, s, OH), 2·18br (1H, s, 4-H), 2·58 (1H, d, *J* 2·5 Hz, 5-H), 2·67br (1H, s, 2-H), 3·22 (1H, d, *J* 2·5 Hz, 7-H), 4·78 (2H, s, CH_2), 6·02 (3H, s, OMe), and 7·85 (3H, s, OAc). Hydrolysis of this compound with aqueous methanolic sodium hydroxide gave teloschistin as lustrous pale orange needles, m.p. 243—244·5° (lit.,[2–4] 229—231, 245—247, 236—237°). Acetylation of teloschistin with acetic anhydride–pyridine gave, after chromatography and crystallisation from methanol, the tri-*O*-acetyl derivative as pale yellow needles, m.p. 194—196° (lit.,[2,4] 192—193°), $\nu_{max.}$ ($CHCl_3$) 1770 (aromatic acetate) and 1740 (aliphatic acetate) cm.$^{-1}$, τ 1·75br (1H, s, 4-H), 2·23 (1H, d, *J* 2·5 Hz, 5-H), 2·53br (1H, s, 2-H), 3·03 (1H, d, *J* 2·5 Hz, 7-H), 4·74 (2H, s, CH_2), 7·54 (6H, s, 2 × nuclear OAc), and 7·82 (3H, s, aliphatic OAc). The slowest moving band was separated and extracted with chloroform, was pre-adsorbed on to silica gel (10 g.), and was chromatographed over a column (2 × 25 cm.) of silicic acid with 5% ethyl acetate–benzene as eluant. The major band on evaporation and crystallisation of the residue from methanol afforded emodin (0·047 g.) as orange needles, m.p. and mixed m.p. with synthetic material (see later) 259—260° (decomp) (lit.,[15] 255°), *m/e* 270 (M^+, 100%), 242 (*M* − CO, *m** 217, calc. 216·9), and 214 (242 − CO, *m** 189, calc. 189·2). The mass and i.r. (KCl) spectra were identical with those of authentic material. The tri-*O*-methyl ether formed fine yellow needles, m.p. 224—226°, and was identical with that prepared from parietin (see before).

(*b*) The thallus (106 g.) was extracted exhaustively with cold chloroform, the extract was evaporated at the pump, and the residue was crystallised from ethyl acetate–light petroleum (b.p. 40—60°) to afford parietin. The mother liquors were concentrated and applied to several layer plates which were developed with chloroform. The front-running band afforded parietin (total 2·03 g.) which was identical with that isolated before. The two slower-running bands were removed in turn and extracted (Soxhlet) with chloroform. The second band gave emodin (1·1 mg.) as orange needles identical (m.p. and t.l.c.) with the product prepared later. The third band gave teloschistin (5·5 mg.) as orange needles identical (m.p. and t.l.c.) with the product already described. The plates were then re-eluted with ethanol, whereupon a fourth band developed. This band was removed and extracted with ethanol–benzene (4 : 1); the extract was concentrated to afford parietinic acid (4·0 mg.) identical (t.l.c. and electronic spectrum) with that already described.

Emodin (II) *by Demethylation of Parietin* (I).—Parietin (57·8 mg.) was maintained at 165° with pyridine hydrochloride (15·5 g.) for 9 hr. The cooled melt was dissolved in dilute hydrochloric acid, and the suspension of quinone was extracted into ethyl acetate. The extract was washed with saturated brine (×2), and dried (Na_2SO_4). T.l.c. of the product showed one spot only. The solvent was removed and the crude product gave orange needles (40·1 mg., 73%), m.p. 259—260° (decomp.) (from methanol) (lit.,[15] 255°).

* Determined in a sealed tube with an electrical coil apparatus and uncorrected.

[20] M. V. Sargent, D. O'N Smith, P. Roffey, and J. A. Elix, *J. Chem. Soc.* (*C*), 1969, 2763.

[21] D. F. G. Pusey and J. C. Roberts, *J. Chem. Soc.*, 1963, 3542.

5-Chloroparietin (XVI).—Parietin (0·532 g.) in the minimum volume (*ca.* 30 ml.) of chloroform was treated with a solution of chlorine (0·146 g.) in chloroform (2·5 ml.) and the solution was stirred in a stoppered flask for 18 hr. The solvent was removed under reduced pressure and the residue (from methanol–chloroform) gave the *chloroquinone* (0·526 g., 88%). A sample sublimed at 180°/0·05 mm. formed fine orange needles, m.p. 220—223° (Found: C, 60·0; H, 3·9. $C_{16}H_{11}ClO_5$ requires C, 60·3; H, 3·5%), τ (CD_2Cl_2) 2·37br (1H, s, 4-H), 2·90br (1H, s, 2-H), 3·20 (1H, s, 7-H), 5·98 (3H, s, OMe), and 7·57 (3H, s, Me). The *methyl ether* formed yellow hexagonal plates from ethyl acetate, m.p. 239—242° (Found: C, 62·6; H, 4·1. $C_{18}H_{15}ClO_5$ requires C, 62·35; H, 4·35%), τ 2·39br (1H, s, 4-H), 2·86br (1H, s, 2-H), 3·21 (1H, s, 7-H), 5·97 (9H, s, OMe), and 7·50 (3H, s, Me).

4,5-Dichloroparietin (XVII).—Parietin (0·407 g.) in chloroform was treated with chlorine (0·266 g.) in chloroform (4 ml.) as already described. T.l.c. of crude product showed two close-running spots, one of which corresponded to the mono-chloroparietin. Separation was achieved by chromatography over four layer plates with benzene as developing solvent. The major orange band was separated and extracted with chloroform. The chloroform was removed and the residue gave pale orange needles (0·241 g., 47·6%), m.p. 249—252° (from chloroform–methanol), of the *dichloro-quinone* (Found: C, 54·15; H, 2·65. $C_{16}H_{10}Cl_2O_5$ requires C, 54·4; H, 2·85%), τ (CD_2Cl_2) −2·90 (1H, s, OH), −2·54 (1H, s, OH), 2·84 (1H, s, 2-H), 3·24 (1H, s, 7-H), 6·04 (3H, s, OMe), and 7·56 (3H, s, Me). The *methyl ether* formed yellow hexagonal plates from chloroform–methanol, m.p. 255—257° (Found: C, 56·9; H, 3·95. $C_{18}H_{14}Cl_2O_5$ requires C, 56·7; H, 3·7%), τ 2·88 (1H, s, 2-H), 3·24 (1H, s, 7-H), 5·99 and 6·02 (total 9H, each s, OMe), and 7·51 (3H, s, Me).

4,5,7-Trichloroparietin (XVIII).—A gentle stream of chlorine was passed through a stirred solution of parietin (0·532 g.) in glacial acetic acid (120 ml.) for 20 min. The flask was then stoppered and set aside for 18 hr., and the excess of chlorine was then removed with a rapid stream of nitrogen. The solvent was removed under reduced pressure and the residue gave orange needles of the *trichloro-quinone* (0·362 g., 41·2%), m.p. 207—209° (from ethanol (Found: C, 49·7; H, 2·35. $C_{16}H_9Cl_3O_5$ requires C, 49·6; H, 2·35%), τ 5·90 (3H, s, OMe) and 7·45 (3H, s, Me); the aromatic proton signal was partially obscured by the chloroform resonance. The *methyl ether* formed pale yellow needles from ethanol, m.p. 231—234° (Found: C, 52·3; H, 3·3. $C_{18}H_{13}Cl_3O_5$ requires C, 52·0; H, 3·15%), τ (CD_2Cl_2) 2·75 (1H, 2-proton), 5·94 and 6·00 (total 9H, each s, OMe), and 7·50 (3H, s, Me).

Dechlorination of 4,5,7-Trichloroparietin (XVIII) *with Sodium Dithionite.*—(*a*) The trichloro-compound (125 mg.) was dissolved in ethanol (20 ml.) and water (20 ml.) containing sodium hydroxide (0·7 g.). Sodium dithionite (3·85 g.) was added and the mixture was stirred under nitrogen for 17 hr. A vigorous stream of oxygen was then passed through the solution for 0·5 hr., and the solution was then acidified with dilute hydrochloric acid. The mixture was exhaustively extracted with ethyl acetate and the extract was washed with water and with saturated brine and dried (Na_2SO_4). The solution was concentrated and applied to two layer plates which were developed with benzene. The major band was separated and extracted with chloroform. The solvent was removed and the residue crystallised from benzene to give parietin (58·6 mg., 62·5%), identical (m.p., t.l.c., and n.m.r. spectrum) with that already described.

(*b*) A solution of sodium hydroxide in water (30 ml.) and methanol (10 ml.) was boiled during the passage of a stream of nitrogen. The solution was allowed to cool, the trichloroparietin (96 mg.) and sodium dithionite (115 mg.) were added, and the solution was stirred for 15 hr. under nitrogen. The total crude product obtained as in (*a*) was methylated and chromatographed over two layer plates with 20% ethyl acetate–benzene as eluant. The faster-moving band afforded 4,5,7-trichlorodi-*O*-methylparietin (28·6 mg.), identical (m.p., mixed m.p., and t.l.c.) with that already described. The slower-moving band yielded tri-*O*-methylemodin (29·1 mg.), identical (m.p., mixed m.p., and t.l.c.) with that already described.

Di-O-methylfragilin (XIX).—(*a*) 4,5,7-Trichloroparietin (195·7 mg.), 10% palladium–charcoal (139 mg.), 99—100% hydrazine hydrate (114 mg.), and ethanol (35 ml.) were boiled under reflux for 0·5 hr. The mixture was filtered and a large excess of dilute hydrochloric acid was added to the filtrate. This mixture was exhaustively extracted with ethyl acetate, and the extract was washed with water and saturated brine, and dried ($MgSO_4$). The residue left after removal of the solvent was methylated, and the crude product was chromatographed over two layer plates with 5% ethyl acetate–benzene as eluant. The major yellow band which developed was separated and extracted with chloroform. The extract was evaporated and the residue was crystallised from methanol to yield the *fragilin* (18·6 mg., 10·6%) as pale yellow needles, m.p. and mixed m.p. with product of (*b*) 212—214° (lit.,[9,12] 217—218, 208—209°); the two samples were identical on t.l.c. in a number of solvent systems (Found: C, 62·55; H, 4·53%; *M*, 346, 348. $C_{18}H_{15}ClO_5$ requires C, 62·35; H, 4·35%; *M*, 346, 348), τ 2·36br (1H, s, 4-H), 2·45 (1H, s, 5-H), 2·93br (1H, s, 2-H), 6·00 and 6·07 (total 9H, each s, OMe), and 7·50 (3H, s, Me).

(*b*) Authentic fragilin (2·1 mg.) was methylated (24 hr.) and the crude product was chromatographed over two t.l.c. plates (20 × 10 × 0·04 cm.) with 5% ethyl acetate–benzene as eluant. The major yellow band was separated and extracted with chloroform, and the extract was evaporated. The residue was crystallised from methanol to give di-*O*-methylfragilin (1·8 mg.) as pale yellow needles, m.p. 212—214°.

Fragilin (IX).—The methyl ether (XIX) (9·0 mg.) was heated under reflux with glacial acetic acid (4 ml.) and 48% aqueous hydrogen bromide (1 ml.) for 1 hr. The solution was diluted with water and exhaustively extracted with ethyl acetate. The extract was washed with water and saturated brine, and dried ($MgSO_4$). The solution was concentrated and chromatographed over two layer plates with benzene as eluant. The major orange band was separated and extracted with chloroform. The solvent was removed and the residue yielded *fragilin* (4·9 mg., 59·3%), m.p. 267—268° (from methanol) (lit.,[8,12] 266—267, 267—268°) identical (mixed m.p., mass spectrum, and t.l.c.) with authentic material (Found: C, 60·1; H, 3·8%; *M*, 318, 320. $C_{16}H_{11}ClO_5$ requires C, 60·3; H, 3·5%; *M*, 318, 320).

7-Chloro-emodin ('AO-1') (VIII).—Di-*O*-methylfragilin (6·1 mg.) was maintained at 160° with pyridine hydro-

chloride (10·8 g.) for 9 hr. The cooled melt was dissolved in water, and the suspension was extracted exhaustively with ethyl acetate. The extract was washed with saturated brine and dried ($MgSO_4$). The solvent was removed and the residue was crystallised from chloroform to give the *chloro-compound* (4·3 mg., 79%). This material showed virtually one spot on t.l.c. but the analytical sample was obtained by chromatography over a layer plate with 20% ethyl acetate–benzene as eluant. The major band was separated and extracted with hot chloroform. The extract was concentrated to afford orange needles, m.p. 281—283° (lit.,[8,9] 271—272, 286—287°), identical (mixed m.p. and t.l.c.) with an authentic sample (Found: *M*, 304·0141. $^{12}C_{15}{}^{1}H_{9}{}^{16}O_{5}{}^{35}Cl$ requires *M*, 304·0138).

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