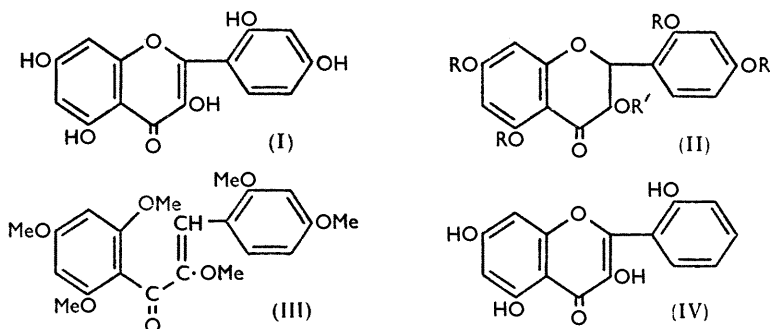


**895. Dihydromorin from East African Mulberry (*Morus lactea* Mildbr.).**

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A crystalline compound isolated from the heartwood of *Morus lactea* has been identified as the hitherto unknown 3 : 5 : 7 : 2' : 4'-pentahydroxyflavanone (dihydromorin).

*Morus lactea* (East African mulberry) is fairly widely distributed in Uganda and occurs also, though only rarely, in Kenya and Tanganyika. The tree has a bole 20—40 feet long and seldom exceeds 6 feet in girth. The timber used in the present investigation was obtained from the Forest Department of Uganda for steam-bending tests, and interest in its extractives first arose when it was observed that, on steaming, the heart-wood became deep yellow throughout. The staining material, which was not found in unsteamed wood, was identified as morin (3 : 5 : 7 : 2' : 4'-pentahydroxyflavone) (I).



A second crystalline compound, isolated from both steamed and unsteamed wood, readily yielded morin on aerial oxidation in aqueous solution,<sup>1</sup> and this, together with elementary analyses, colour reactions, and ultraviolet absorption data<sup>2-5</sup> (see Figs. 1 and 2) indicated that the compound was dihydromorin (3 : 5 : 7 : 2' : 4'-pentahydroxyflavanone) (II; R = R' = H).

Acetylation yielded a penta-acetate, confirming the presence of five hydroxyl groups, four of which were shown to be phenolic by the formation of a tetramethyl ether (II;

<sup>1</sup> Pew, *J. Amer. Chem. Soc.*, 1948, **70**, 3031.

<sup>2</sup> Skarzynski, *Biochem. Z.*, 1939, **301**, 150.

<sup>3</sup> Hergert, Coad, and Logan, *J. Org. Chem.*, 1956, **21**, 304.

<sup>4</sup> Geissman and Lischner, *J. Amer. Chem. Soc.*, 1952, **74**, 3001.

<sup>5</sup> Hillis, *Austral. J. Sci. Res.*, 1952, **5**, A, 379.

R = Me, R' = H) and its monoacetate (II; R = Me, R' = Ac). Treatment of dihydromorin with diazomethane produced a mixture of partly methylated derivatives. Low-temperature methylation under strongly alkaline conditions gave a product (H) which was similar to the hexamethoxychalkone prepared<sup>3</sup> from dihydroquercetin and may be 2 : 2' : 4 : 4' : 6' :  $\alpha$ -hexamethoxychalkone (III). This material was not examined further at this stage.

It has been shown elsewhere<sup>6</sup> that a chalkone with a 6'-methoxyl group lacking a hydroxyl in the 2- or 4-position gives mainly aurone, and not a flavonol or dihydroflavonol, on alkaline peroxide oxidation. In order to avoid this difficulty, the preparation of 2 : 2' : 4-trihydroxy-4' : 6'-dimethoxychalkone as an intermediate for the synthesis of the corresponding dihydroflavonol was attempted, but without success. Formation of the known 2'-hydroxy-2 : 4 : 4' : 6'-tetramethoxychalkone<sup>7</sup> proceeded smoothly, however, and alkaline peroxide oxidation of this material gave dihydro-5 : 7 : 2' : 4'-tetra-O-methylmorin in small yield, thus confirming the structure of dihydromorin. The synthetic product was slightly impure and appeared to contain some tetra-O-methylmorin, which probably prevented the ready formation of a crystalline acetate. No attempt was made to isolate any aurone formed during the oxidation (cf. Geissman and Fukushima<sup>6</sup>).

Treatment of morin with alkaline dithionite under a variety of conditions<sup>1,4,8</sup> failed to produce any appreciable amount of dihydromorin, whereas quercetin<sup>1,4</sup> and kempferol<sup>5</sup> are readily reduced by this method to their dihydro-derivatives. This anomaly may be due to some steric effect associated with the 2'-hydroxyl group, particularly as datiscetin (5 : 7 : 2'-trihydroxyflavonol) (IV) also resisted reduction.

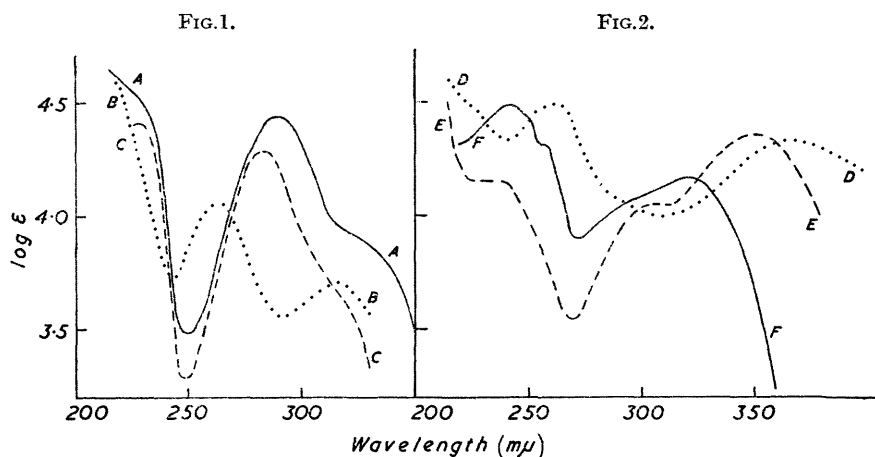


FIG. 1. Absorption spectra of (A) dihydromorin, (B) dihydromorin penta acetate, and (C) dihydro-5 : 7 : 2' : 4'-tetra-O-methylmorin.

FIG. 2. Absorption spectra of (D) morin, (E) methylated product (H), and (F) penta-O-methylmorin.

Dr. C. H. Lea, of the Low Temperature Research Station, Cambridge, finds that dihydromorin has no appreciable antioxidant activity,<sup>9</sup> in accord with expectation.

Several naturally occurring and synthetic dihydroflavonols are known<sup>10</sup> but the identification of dihydromorin in *Morus lactea* is of special interest, as this is the first example of a dihydroflavonol containing a 2'-hydroxyl group yet isolated. Further, the

<sup>6</sup> Geissman and Fukushima, *J. Amer. Chem. Soc.*, 1948, **70**, 1686; Anand, Iyer, and Venkataraman, *Proc. Indian Acad. Sci.*, 1949, **29**, A, 203; Simpson and Whalley, *J.*, 1955, 166.

<sup>7</sup> Kostanecki and Tambor, *Ber.*, 1904, **37**, 792.

<sup>8</sup> Shimizu and Yoshikawa, *J. Pharm. Soc. Japan*, 1952, **72**, 331.

<sup>9</sup> Lea and Swoboda, *Chem. and Ind.*, 1956, 1426; Simpson and Uri, *ibid.*, p. 956.

<sup>10</sup> Gowan, Philbin, and Wheeler, "The Chemistry of Vegetable Tannins: A Symposium held at Cambridge, 1956," Society of Leather Trades' Chemists, Croydon, 1956, p. 133.

unsteamed timber contained no appreciable amount of morin, though this compound has been reported<sup>11</sup> to be present in other timbers of the family Moraceae (*Chlorophora tinctoria*, *Maclura pomifera*, *Artocarpus integrifolia*, *Morus alba*).

#### EXPERIMENTAL

The wood, obtained from the Uganda Forest Department, was received as pieces 2' × 3' × 3' which had been steamed and bent. Only a very small amount of unsteamed wood was available. The pale yellow heartwood alone was used for extraction, it being readily distinguishable from the pale sapwood which was shown to contain little or no dihydromorin.

Paper chromatography was carried out on Whatman's No. 1 paper by the descending method, and the following solvent systems<sup>12</sup> were used: (A) 15% aqueous acetic acid; (B) butan-1-ol-acetic acid-water (4 : 1 : 5) (unbuffered); (C) 22% aqueous isopropyl alcohol. The positions of the spots on the chromatogram were established by examination under ultraviolet light, morin giving a yellow fluorescent spot and dihydromorin a brown spot, and also by spraying with diazotised *p*-nitroaniline,<sup>13</sup> morin giving a brown colour and dihydromorin a bright orange-brown colour.  $R_F$  values were calculated from the distance travelled by the forward edge of the spot, as revealed under ultraviolet light.

Authentic morin, obtained from L. Light and Co. Ltd., contained small amounts of impurities which were removed by extraction with boiling water, followed by recrystallisation from dilute acetic acid. The product appeared to be chromatographically homogeneous.

Ultraviolet absorption spectra were determined in methanol solution in a Unicam S.P. 500 spectrophotometer.

The light petroleum used throughout had b. p. 60–80°, unless otherwise stated.

*Extraction of Morin.*—Sawdust from the steamed wood was extracted with water and morin precipitated from the extract as the lead salt.<sup>14</sup> The precipitate was decomposed, and the product, after recrystallisation from aqueous ethanol, was purified by passage through a column of cellulose<sup>15</sup> (solvent A). Morin crystallised from the eluate as pale yellow needles, m. p. and mixed m. p. 302° (decomp.), in very low yield. This material had the same  $R_F$  value on the paper chromatogram as an authentic sample run on the same paper (solvents A, B, and C).

*Extraction of Dihydromorin.*—(a) Sawdust from steamed wood (1009 g., dry wt.) was extracted with ether (Soxhlet) for 16 hr. Evaporation of the extract under reduced pressure gave a solid residue which was extracted with boiling water (charcoal). The mixture was filtered and the filtrate, on cooling, deposited colourless crystals of dihydromorin (8.08 g., 0.8%) as the hydrate, m. p. 228° (loses water of crystallisation at 147°). Anhydrous dihydromorin was obtained by recrystallisation from ether-light petroleum (b. p. 40–60°) as clusters of needles, m. p. 228°,  $[\alpha]_D^{20} \pm 0^\circ$  (*c* 1.0 in 1 : 1 Me<sub>2</sub>CO-H<sub>2</sub>O) (Found: C, 59.0; H, 4.0. C<sub>15</sub>H<sub>12</sub>O<sub>7</sub> requires C, 59.2; H, 3.95%). It was soluble in acetone, ethanol, methanol, ether, and aqueous sodium hydroxide, sodium carbonate, and sodium hydrogen carbonate, and insoluble or only very slightly soluble in light petroleum and in benzene. It gave a red-brown ferric colour in ethanol, a red colour with both magnesium-hydrochloric acid and zinc-hydrochloric acid,<sup>1</sup> and a negative boric acid test.<sup>16</sup> On the paper chromatogram it had  $R_F$  0.60 (solvent A), 0.92 (solvent B), 0.68 (solvent C). Morin on the same paper had  $R_F$  0.25, 0.95, and 0.37 respectively. When the compound was applied to the chromatogram in aqueous solution, and especially with heat, two spots were obtained in solvents A and C owing to the formation of morin by aerial oxidation.

(b) Unsteamed wood (51.5 g.) was extracted with ether as above. Evaporation yielded a solid residue (0.58 g.) in which no morin could be detected on the chromatogram, though dihydromorin was shown to be present. Recrystallisation yielded dihydromorin (0.22 g., 0.43%), m. p. and mixed m. p. 224°,  $R_F$  as above,  $[\alpha]_D^{20} \pm 0^\circ$  (*c* 0.6 in 1 : 1 Me<sub>2</sub>CO-H<sub>2</sub>O).

*Oxidation of Dihydromorin.*—Dihydromorin (1.25 g.) was dissolved in hot water (65 c.c.) in

<sup>11</sup> Wise and Jahn, "Wood Chemistry," Reinhold Publ. Corp., New York, 1952, Vol. I, p. 628 *et seq.*; Barnes and Gerber, *J. Amer. Chem. Soc.*, 1955, **77**, 3259; Suzushino, *Misc. Reports Res. Inst. Nat. Resources (Japan)*, 1954, No. 34, 21; *Chem. Abs.*, 1957, **51**, 1396.

<sup>12</sup> Gage, Douglass, and Wender, *Analyt. Chem.*, 1951, **23**, 1582.

<sup>13</sup> Swain, *Biochem. J.*, 1953, **53**, 200.

<sup>14</sup> Perkin and Everest, "The Natural Organic Colouring Matters," Longmans, Green and Co., London, 1918, p. 213.

<sup>15</sup> Hough, Jones, and Wadman, *J.*, 1949, 2511.

<sup>16</sup> Wilson, *J. Amer. Chem. Soc.*, 1939, **61**, 2303.

an open flask and the solution was heated on a steam-bath with frequent shaking, water being added periodically to replace that lost by evaporation. At 8-hourly intervals, the morin which separated was removed by filtration. The total yield, after 40 hours' heating, was 50%, and the m. p. after recrystallisation from 33% acetic acid was 298° (mixed m. p. 302°) (Found: C, 59.7; H, 3.4. Calc. for  $C_{15}H_{10}O_7$ : C, 59.6; H, 3.3%). This showed the same  $R_F$  value on the paper chromatogram as authentic morin (solvents A and C).

The product (0.28 g.) was methylated with methyl sulphate in acetone,<sup>17</sup> to give penta-*O*-methylmorin, m. p. and mixed m. p. 157—158° (Found: OMe, 39.8. Calc. for  $C_{20}H_{20}O_7$ : OMe, 41.7%).

The ultraviolet absorption spectra of the morin and penta-*O*-methylmorin prepared in this manner (Fig. 2) corresponded closely to those of authentic samples.

*Acetylation of Dihydromorin.*—Dihydromorin (0.2 g.) with acetic anhydride (2 c.c.) in pyridine (10 c.c.) gave penta-*O*-acetyldihydromorin (0.14 g. after two recrystallisations from methanol), as white needles, m. p. 190—193°,  $[\alpha]_D^{20} \pm 0^\circ$  (*c* 1.0 in  $Me_2CO$ ) (Found: C, 58.3; H, 4.5; Ac, 39.5.  $C_{25}H_{22}O_{12}$  requires C, 58.35; H, 4.3; Ac, 41.8%).

*Methylation with Diazomethane.*—Dihydromorin in ether was treated with excess of ethereal diazomethane at 0° for 3 days. The solid product (Found: OMe, 12.8%) was recrystallised from benzene–light petroleum and had m. p. 173° (decomp.). Paper-chromatography with butanol–ethanol–water (4 : 1 : 5, top layer) and benzene–pyridine–water (100 : 1 : 100, top layer)<sup>18</sup> showed it to be a mixture in which two components (probably mono- and di-*O*-methyl derivatives) predominated.

*Dihydro-5 : 7 : 2' : 4'-tetra-*O*-methylmorin.*—Dihydromorin (1 g.) in acetone was methylated with methyl sulphate in the presence of anhydrous potassium carbonate,<sup>3</sup> and the product was recrystallised first from aqueous ethanol and then twice from ethanol–light petroleum to give dihydro-5 : 7 : 2' : 4'-tetra-*O*-methylmorin (0.57 g.) as white, feathery needles, m. p. 188—190° (Found: C, 63.5; H, 5.75; OMe, 31.1.  $C_{19}H_{20}O_7$  requires C, 63.3; H, 5.6; OMe, 34.4%). The compound gave a red colour with zinc and hydrochloric acid<sup>1</sup> and no colour with ferric chloride in ethanol.

Acetylation of this material gave 3-*O*-acetyldihydro-5 : 7 : 2' : 4'-tetra-*O*-methylmorin, white prisms (from methanol), m. p. 168° (Found: C, 62.3; H, 5.2; Ac, 10.4.  $C_{21}H_{22}O_8$  requires C, 62.7; H, 5.5; Ac, 10.7%).

*Low-temperature Methylation of Dihydromorin.*—Dihydromorin (1 g.) was dissolved in acetone (15 c.c.), and methyl sulphate (10 c.c.) and potassium hydroxide (9 g. in 15 c.c. aqueous solution) were added simultaneously during 3½ hr. with constant stirring, under nitrogen, at  $> 0^\circ$ . The mixture was then acidified and the precipitate remethylated as above. The final product was a viscous oil which was washed with water and crystallised from ethanol–light petroleum, to give pale yellow plates of substance (H) (0.14 g.), m. p. 117—118° (Found: C, 64.5; H, 6.35; OMe, 45.6.  $C_{23}H_{24}O_7$  requires C, 64.9; H, 6.2; OMe, 47.9%). The compound gave no colour with ferric chloride and an orange colour with alcoholic hydrochloric acid. The ultraviolet absorption spectrum is shown in Fig. 2; no shift was obtained with sodium methoxide or aluminium chloride.<sup>19</sup>

*2'-Hydroxy-2 : 4 : 4' : 6'-tetramethoxychalkone.*—2-Hydroxy-4 : 6-dimethoxyacetophenone (1.25 g.) and 2 : 4-dimethoxybenzaldehyde (1.02 g.) were dissolved in ethanol (25 c.c.) and sodium hydroxide (3 g.) in water (6 c.c.) was added. The mixture was left overnight at room temperature, then acidified with hydrochloric acid. The precipitated chalkone was recrystallised from ethanol [yield, 1.6 g., m. p. 150—152° (lit.,<sup>7</sup> 152°)] (Found: C, 65.9; H, 5.9; OMe, 35.8. Calc. for  $C_{19}H_{20}O_6$ : C, 66.3; H, 5.9; OMe, 36.0%).

*3-Hydroxy-5 : 7 : 2' : 4'-tetramethoxyflavanone.*—The foregoing chalkone (3 g.) was suspended with shaking in 5% aqueous sodium hydroxide (450 c.c.), and 100-vol. hydrogen peroxide (25 c.c.) added. The mixture was left overnight at 0°. Unchanged chalkone and, presumably, any aurone formed<sup>6</sup> were filtered off, the filtrate was acidified, and the precipitated material was collected. The chalkone was re-treated twice as above and the products were combined and recrystallised from dilute acetic acid, washed with ether, and finally recrystallised from ethanol–light petroleum. It (0.053 g.) had m. p. 179°, mixed m. p. with dihydro-5 : 7 : 2' : 4'-tetra-*O*-methylmorin prepared by direct methylation of dihydromorin, 183—186°. It gave a red

<sup>17</sup> Jain, Seshadri, and Thiruvengadam, *Proc. Indian Acad. Sci.*, 1952, **36**, A, 217.

<sup>18</sup> Simpson and Garden, *J.*, 1952, 4638.

<sup>19</sup> Harborne, *Chem. and Ind.*, 1954, 1142; Swain, *ibid.*, p. 1480.

colour with zinc and hydrochloric acid, and a green colour with ferric chloride in ethanol, indicating the presence of some tetra-*O*-methylmorin (Found: C, 63.2; H, 5.3; OMe, 34.1. Calc. for  $C_{18}H_{20}O_7$ : C, 63.3; H, 5.6; OMe, 34.4%).

Attempts to prepare a crystalline acetate were unsuccessful.

*Other Experiments.*—Attempts to prepare 2:2':4-trihydroxy-4':6'-dimethoxychalkone from 2-hydroxy-4:6-dimethoxyacetophenone and 2:4-dihydroxybenzaldehyde by the above method or by heating the mixture under nitrogen<sup>20</sup> were unsuccessful.

Attempted synthesis of the dihydrotetramethoxyflavonol *via* the corresponding *o*-acetoxychalkone dibromide<sup>21</sup> was unsuccessful.

Suspension of the 2'-hydroxy-2:4:4':6'-tetramethoxychalkone in alcohol followed by alkaline oxidation<sup>21</sup> furnished some dihydrotetramethoxyflavonol (positive zinc-hydrochloric acid test), but the yield was very small and the product could not be purified.

*Attempted Reduction of Datiscetin.*—The datiscetin used had m. p. 277° and  $R_F$  0.42 (solvent A), 0.96 (solvent B), 0.30 (solvent C).  $R_F$  values for morin, run on the same papers, were 0.30, 0.94, and 0.22 respectively. Reduction of this material (0.2 g.) with dithionite failed to give any appreciable quantity of the dihydro-derivative, while a parallel reduction of quercetin gave dihydroquercetin in good yield.

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<sup>20</sup> Guider, Simpson, and Thomas, *J.*, 1955, 170.

<sup>21</sup> Marathe, *J. Univ. Poona, Science and Technology*, 1953, 4, 73.

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