

**442. Chlorophyll and Related Compounds. Part V.\* The Dihydrohæmatinic Acids and their Imides.**

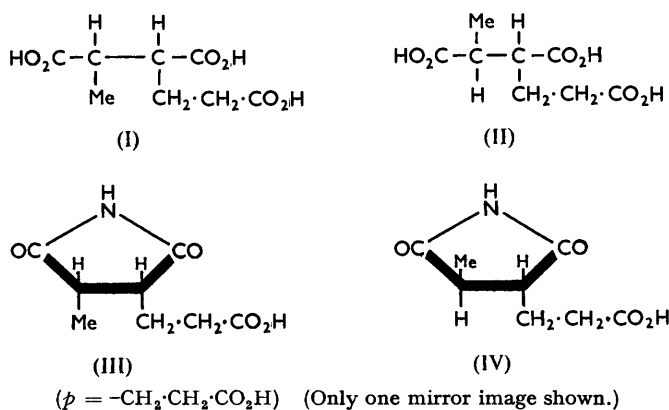
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The two diastereoisomeric forms of dihydrohæmatinic acid, and their imides, have been prepared and characterised. Their interconversions have been studied in relation to those in the  $\alpha\alpha'$ -dimethylsuccinic series. Probable configurations are assigned.

IN connection with the work described in the preceding paper it was necessary to make a full investigation of the stereoisomeric dihydrohæmatinic acids and their imides. These compounds have been the subject of a valuable study by Küster<sup>1</sup> but the literature was incomplete.

Our reinvestigation of these substances is described in the Experimental section. Previous synthetic work has already been reviewed in the preliminary account which we have communicated to the Society<sup>2</sup> and it is only necessary here to summarise the main conclusions.

There are two diastereoisomeric dihydrohæmatinic acids (I and II) which yield the corresponding imides (III and IV).† All four compounds have now been obtained



crystalline and the two imides yield crystalline benzylamine salts and solid disilver derivatives. Unless extremely pure the free imides show a reluctance to crystallise and the benzylamine salts are convenient for their isolation and purification. Compounds of

\* Part IV, preceding paper.

† The imides still contain one carboxyl group and have sometimes been referred to as "acids", but to avoid ambiguity we think it best to restrict the name "dihydrohæmatinic acid" to the tricarboxylic acids. The triacids have also been given the trivial names dihydrohæmatic acid and hæmotricarboxylic acid.

<sup>1</sup> Particularly *Annalen*, 1906, **345**, 1.

<sup>2</sup> Linstead, Eisner, Ficken, and Johns, *Chem. Soc. Special Publ.*, No. 3, 1955.

the two series have distinct melting points (see Table), infrared spectra, and (under suitable conditions) different chromatographic behaviour.

Configuration Formulae	Series 1	Series 2
	<i>cis</i> - or <i>cisoid</i> -* (I) and (III)	<i>trans</i> - or <i>transoid</i> -* (II) and (IV)
M. p. : acid .....	176°	143°
imide .....	124	85
benzylamine salt of imide .....	160	163

\* *cis*- and *trans*- refer to the disposition of the hydrogen atoms about the plane of the heterocyclic ring in (III) and (IV); *cisoid*- and *transoid*- refer to the corresponding open-chain compounds.

The configurations can be assigned with some confidence from the analogy with the  $\alpha'$ -dimethylsuccinic acids.<sup>3</sup> In particular, (a) the imide of m. p. 124° is obtained by the catalytic hydrogenation of hæmatinic imide under conditions which give *meso*(*cis*)-dimethylsuccinimide from dimethylmaleimide; (b) the imide of m. p. 124° gives its *trans*-isomer when heated with urea, which recalls the formation of *trans*-dimethylsuccinimide by fusion of the *cis*-anhydride with urea; (c) the anhydride of the *trans*-series is the thermally stable form, and a pure *cis*-anhydride has not been isolated.

The inversion of the triacids, by means of water at 200°, proceeds in the direction *cisoid*  $\rightarrow$  *transoid*, and may involve intermediate formation of the anhydrides. The *cisoid*-acid is slowly inverted in boiling hydrochloric acid. Inversion of the acids with baryta at 225° is slow and reversible.

#### EXPERIMENTAL

Microanalyses are by Mr. Oliver, infrared spectra by Mr. Erskine of this Department.

*Preparation of the Dihydrohæmatinic Acids.*—(a) Diethyl  $\alpha$ -cyano- $\alpha'$ -methylsuccinate was prepared from ethyl cyanoacetate and ethyl  $\alpha$ -bromopropionate, following Küster's directions,<sup>4</sup> in 81% yield, and had b. p. 173—176°/30 mm. The  $\beta$ -propionic acid residue was introduced either by condensing the sodio-derivative of this product with ethyl  $\beta$ -chloropropionate, as was done by Küster,<sup>4</sup> or with  $\beta$ -chloropropionitrile. In the latter modification, a mixture of diethyl  $\alpha$ -cyano- $\alpha'$ -methylsuccinate (39.5 g.) and  $\beta$ -chloropropionitrile (18.1 g.) was added to a solution of sodium (4.3 g.) in ethanol (100 ml.) at 0°. After 19 hours' heating on the steam-bath the product was worked up in the usual manner. The yield of diethyl  $\alpha$ -cyano- $\alpha$ -2-cyanoethyl- $\alpha'$ -methylsuccinate was 30.8 g. (63%), and the b. p. 187—192°/0.8 mm. On refractionation this had b. p. 189°/0.8 mm.,  $n_D^{19}$  1.4563 (Found: C, 58.8; H, 6.8; N, 10.5.  $C_{13}H_{18}O_4N_2$  requires C, 58.6; H, 6.8; N, 10.5%).

When the original Küster procedure was followed, 133 g. of the product were refluxed for 50 hr. with 450 ml. of concentrated hydrochloric acid. The product was concentrated to 300 ml. and cooled; the solid was collected. The mother-liquor was extracted continuously with ether for several days. The ether deposited a further crop of solid. The two solids were combined and crystallised repeatedly from acetone, which yielded *cisoid*-dihydrohæmatinic acid (I), m. p. 176—178° (24.4 g.). The ether extract after removal of the solid was evaporated to dryness and the residue heated at 200° for 3 hr. The product was boiled with dilute hydrochloric acid (200 ml.) and charcoal, and the solution filtered and evaporated to dryness. The product after repeated crystallisation from acetone yielded *transoid*-dihydrohæmatinic acid (II), m. p. 143—144° (14.3 g.). Similar results were obtained on hydrolysis of diethyl  $\alpha$ -cyano- $\alpha$ -2-cyanoethyl- $\alpha'$ -methylsuccinate.

(b) Hæmatinic acid (495 mg.) was hydrogenated as a solution of its neutral sodium salt in aqueous ethanol over a palladium-charcoal, 1 mol. of hydrogen being taken up. The product was acidified, the catalyst and solvent were removed, and the acid was isolated by continuous ether-extraction. Removal of the ether and crystallisation from benzene-ethyl acetate-methanol gave *cisoid*-dihydrohæmatinic acid (196 mg.), needles, m. p. 176°.

The maxima in the infrared spectra of the two acids (Nujol) are:

176° isomer: 2667, 1787 vw, 1705, 1691 s, 1408, 1344, 1321, 1314, 1289, 1258, 1233, 1218, 1199, 1186, 1131, 1095, 1076, 1048, 1027, 981, 951, 935, 910, 861, 853, 804, 766, 753, 722, and 695  $cm^{-1}$ .

<sup>3</sup> Linstead and Whalley, *J.*, 1954, 3722.

<sup>4</sup> Küster, *Z. physiol. Chem.*, 1923, 130, 1.

144° isomer : 2732, 2674 s, 1706, 1678 s, 1427, 1406, 1353, 1325, 1295, 1272, 1250 s, 1238 s, 1209 m, 1166, 1147, 1079, 1059, 1027, 995, 952, 939, 891, 851, 803, 737, 721, and 671 cm.<sup>-1</sup>.

*trans-Dihydrohæmatinic Anhydride*.—The acid of m. p. 144° (1.74 g.) was heated at 0.5 mm. At 150° the white *anhydride* sublimed rapidly (1.01 g.; m. p. 94—95°) (Found : C, 51.2; H, 5.6. C<sub>8</sub>H<sub>10</sub>O<sub>5</sub> requires C, 51.6; H, 5.4%). The acid, m. p. 176°, was heated at 180—185° for 3 hr. and then sublimed at 140°/0.1 mm. The sublimate melted at 84—90°, not depressed by admixture with the product of m. p. 94—95°. Hydrolysis with dilute hydrochloric acid yielded impure *transoid*-acid, m. p. 131—136°, not depressed on admixture with the acid of m. p. 144°.

When the acid of m. p. 176° was refluxed for 15 hr. with acetyl chloride and the reagent removed, an oil was obtained. It was probably the *cis*-anhydride, or a mixed anhydride with acetic acid, as it regenerated the *cisoid*-acid when warmed with hydrochloric acid. Use of acetic anhydride in place of acetyl chloride led to inversion and the regenerated acid melted at 135—138°, not depressed by the acid of m. p. 144°.

*Inversion of the Acids*.—The acid, m. p. 176° (534 mg.), was heated with water for 5 hr. at 200° (Carius tube). Evaporation and crystallisation gave the *transoid*-isomer (470 mg.; m. p. 140—145°; no depression with the acid of m. p. 144°). The acid, m. p. 176°, was, however mainly unchanged by an excess of barium hydroxide at 225° (5 hr.). The yield of inverted acid was under 20%. The acid of m. p. 144° was similarly treated with barium hydroxide, and the product isolated by acidification and ether-extraction. The residue from the first extract melted at 155—159°. Subsequent continuous extraction yielded unchanged acid of m. p. 144°. From mixed m. p. curves the inversion 144° → 176° is estimated to be about 15%.

*Dihydrohæmatinic Imides*.—(a) *trans-Isomer* (IV). *cisoid*-Dihydrohæmatinic acid, m. p. 176° (6.76 g.), was heated for 80 min. at 155—160° with urea (2.50 g.). The product was cooled, treated with water (20 ml.) and concentrated hydrochloric acid (8 ml.), and continuously extracted with ethyl acetate. The extract on evaporation to dryness left an almost colourless oil which slowly solidified in a vacuum-desiccator (4.70 g.). The imide yielded a *benzylamine salt* which was prepared in ethyl acetate and crystallised from ethyl acetate-ethanol (overall yield 65% on the original triacid); it had m. p. 163—164° (Found : C, 61.8; H, 6.9; N, 9.6. C<sub>15</sub>H<sub>20</sub>O<sub>4</sub>N<sub>2</sub> requires C, 61.6; H, 6.9; N, 9.6%). The infrared spectrum of the salt (Nujol) showed maxima in the region 690—1300 cm.<sup>-1</sup> at 1293, 1262, 1215, 1188 s, 1166 m, 1156 m, 1093, 1059, 981, 949, 909, 889, 873, 823, 794, 776, 753 s, 741, 721, and 696 s cm.<sup>-1</sup>. Complete hydrolysis of the benzylamine salt with boiling hydrochloric acid gave the *transoid*-dihydrohæmatinic acid in good yield and purity.

The benzylamine salt (3.13 g.), dissolved in the minimum amount of water, was treated with cold concentrated hydrochloric acid (1.5 ml.), and the solution extracted with ethyl acetate (3 × 50 ml.). The extracts were dried and freed from solvent. The residual oil (1.93 g., 95%) slowly solidified in a vacuum-desiccator. After crystallisation from ethyl acetate-benzene the pure *trans-imide* [*trans-β*-(4-methyl-2 : 5-dioxo-3-pyrrolidinyl)propionic acid] (IV), m. p. 85—87°, was obtained (Found : C, 51.9; H, 5.9; N, 7.4. C<sub>8</sub>H<sub>11</sub>O<sub>4</sub>N requires C, 51.9; H, 6.0; N, 7.6%). The infrared spectrum (Nujol) showed maxima at 3086, 2962, 1774, 1703, 1404, 1353, 1319, 1287, 1243, 1204, 1188, 1116, 1104, 1065, 1051, 1020, 957, 907, 835, 789, 741, 720, and 697 cm.<sup>-1</sup>.

The crude imide could be sublimed slowly 150°/0.2 mm. but this failed to give a homogeneous product. The m. p. of the sublimate was 52—65°. By the procedure given by Fischer and Wenderoth<sup>5</sup> the imide was converted into a *disilver derivative* (Found : C, 24.0; H, 2.5; N, 3.5; Ag, 53.8. C<sub>8</sub>H<sub>9</sub>O<sub>4</sub>NAg<sub>2</sub> requires C, 24.1; H, 2.3; N, 3.5; Ag, 54.1%). From this, acidification with hydrochloric acid and extraction with ethyl acetate regenerated the imide but there was some inversion in the total process and the m. p. sank to 49—65°.

The imide was also characterised as the *S-benzylthiuronium salt*, m. p. 150—151° after crystallisation from aqueous ethanol (Found : C, 54.7; H, 6.2; N, 12.0. C<sub>16</sub>H<sub>21</sub>O<sub>4</sub>N<sub>3</sub>S requires C, 54.7; H, 6.0; N, 12.0%).

When *transoid*-dihydrohæmatinic acid was fused with urea under the same conditions (at 155—160°) the overall yield of imide benzylamine salt was only 6.5%; but when the fusion temperature was raised to 175—180°, the yield of imide benzylamine salt was raised to 46%. The product melted at 157—159°, not depressed by the compound already described.

(b) *cis-Isomer* (III). Hæmatinic imide was prepared following Muir and Neuberger's directions<sup>6</sup> except that a lower temperature (140—145°) was found preferable for the urea fusion. The *benzylamine salt*, crystallised from ethyl acetate-ethanol, had m. p. 155° (Found : C, 62.2; H, 6.5; N, 9.8. C<sub>15</sub>H<sub>18</sub>O<sub>4</sub>N<sub>2</sub> requires C, 62.1; H, 6.3; N, 9.7%). Ultraviolet

<sup>5</sup> Fischer and Wenderoth, *Annalen*, 1940, **545**, 140.

<sup>6</sup> Muir and Neuberger, *Biochem. J.*, 1949, **45**, 164.

absorption max. (in EtOH) at 222  $m\mu$  ( $\log \epsilon$  4.22). Infrared bands (Nujol) at: 2735 s, 2650 m, 1761 w, 1709 w, 1677 sh, 1618 m, 1534, 1414 s, 1342 s, 1317 m, 1280, 1277, 1216, 1182, 1153, 1086, 1070, 1027, 1006, 973, 942, 930, 916, 897, 865, 826, 793, 781, 754, 747, 726, and 696  $cm^{-1}$ .

*Hydrogenation*.—A solution of hæmatinic imide (840 mg.) in methanol (10 ml.) was hydrogenated at room temperature over 10% palladium-charcoal catalyst (96 mg.); 0.96 mol. of hydrogen was absorbed. The filtered solution was evaporated to dryness in a vacuum-desiccator and gave a quantitative yield of *dihydrohæmatinic imide*, m. p. 106—119°, which after crystallisation from ethyl acetate had m. p. 124—126° (Found: C, 51.8; H, 6.1; N, 7.6.  $C_8H_{11}O_4N$  requires C, 51.9; H, 6.0; N, 7.6%). Infrared max. (Nujol) at: 3175 m, 2728 w, 2611 w, 1764 w, 1707 w, 1541 w, 1420, 1362 s, 1323 m, 1302, 1277 m, 1232 m, 1204 s, 1185 s, 1144, 1106, 1074, 1055, 1022, 992, 944, 894, 881, 866, 805, 773, and 739  $cm^{-1}$ . The use of Adams catalyast led to a less pure product, m. p. 92—105°.

The *cis*-imide was characterised by the preparation of the *disilver derivative* (Found: C, 23.9; H, 2.4; N, 3.5; Ag, 54.1.  $C_8H_9O_4NAg_2$  requires C, 24.1; H, 2.3; N, 3.5; Ag, 54.1%), and the *benzylamine salt*. The latter was obtained in 92% yield in ethyl acetate solution, and crystallised from ethanol (Found: C, 61.5; H, 7.0; N, 9.7.  $C_{15}H_{20}O_4N_2$  requires C, 61.6; H, 6.9; N, 9.6%). The m. p., 160—161°, was not depressed on admixture with the benzylamine salt of the isomeric imide but the infrared spectrum was distinct, *viz.*, max. (Nujol) at: 2745 s, 2650, 2200, 1771, 1719 s, 1623, 1527 s, 1418, 1402 s, 1346 s, 1324, 1290, 1210, 1194, 1159, 1098, 1067, 1040, 984, 954, 922, 896, 891, 869, 837, 786, 763, 741, 722, and 695  $cm^{-1}$ . The same salt was obtained by catalytic hydrogenation of the benzylamine salt of hæmatinic imide.

Treatment of the *cis*-benzylamine salt with cold hydrochloric acid (as described for the *trans*-isomer) regenerated the *cis*-imide in excellent yield and purity (m. p. 125—126°). Hydrolysis of the *cis*-imide with boiling hydrochloric acid for 16 hr. gave mainly *cisoid*-acid, there having been some inversion. After crystallisation from water the acid melted at 161—168°. A similar mixture was obtained by treating the pure *cisoid*-acid under the same conditions.

The *cis*-imide was fused with urea for 105 min. at 160—165°. The product, isolated in the manner described above for other urea fusions, was an oil. It was converted into a benzylamine salt, m. p. 162°, which was shown to be that of the *trans*-isomer by the identity of the infrared spectra.

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