CCXCIV. S-CYSTEINOSUCCINIC ACID

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WE have reported previously [Morgan & Friedmann, 1938, 1] that thiol compounds react with maleic acid giving stable condensation products of the general formula $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}(\text{COOH}) \cdot \text{SR}$. Of the three substances investigated, thiolacetic acid, *l*-cysteine and glutathione, only thiolacetic acid gave an easily crystallizable reaction product. We have now succeeded in crystallizing the product obtained by the interaction of *l*-cysteine with maleic acid.

The new substance which we call *S-cysteinossuccinic acid* separates from methanol in hedgehog crystals, m.p. 134–135° after softening at 102° and from glacial acetic acid in needles, softening at 125° and melting at 134–135°. The analytical figures of both products were in agreement with the formula $\text{C}_7\text{H}_{11}\text{O}_6\text{NS}$, but the optical rotations were different. The substance from methanol was laevorotatory in aqueous solution whilst that from glacial acetic acid was optically inactive. It was found in fact that hot glacial acetic acid induced partial or complete racemization; thus the optical rotation of the laevorotatory substance from methanol ($[\alpha]_D = -29.8^\circ$), after one recrystallization from glacial acetic acid decreases considerably ($[\alpha]_D = -5.1^\circ$) and becomes zero after a second recrystallization from the same solvent. Simultaneously the form of the crystals changes from rosettes to grid needles.

The addition of *l*-cysteine to maleic acid involves the formation of a second asymmetric centre (**):

so that two optically active condensation products can result. So far we have no experimental evidence for the actual formation of a second optically active compound. The laevorotatory substance described in this paper is certainly the main, if not the only, reaction product. It can be isolated in a yield of 86.75% from the amorphous product resulting (93.5% yield) from the interaction of *l*-cysteine with maleic acid [Morgan & Friedmann, 1938, 1].

The directing influence of the first asymmetric carbon atom (*) on the configuration of the newly formed second asymmetric carbon atom (**) is in agreement with well known stereochemical observations. The actual configuration of this second asymmetric carbon atom (**) however must at present remain open.

The maleic acid reaction has been applied by us [Morgan & Friedmann, 1938, 2] to the study of enzyme reactions induced by SH-compounds. In the discussion of these results we pointed out that the conclusions based on the inhibition of an enzyme reaction by maleic acid must be checked by a direct method. We suggested Hopkins's SS, SH reaction [Hopkins, 1921] as suitable for this purpose [Hopkins & Morgan, 1938]. Recently v. Euler & Hellström [1938] have raised objections against the validity of conclusions based on Hopkins's reaction. We tried to supply the missing direct chemical evidence for an assumed interaction of maleic acid with SH-groups of enzymes or coenzymes. As SH occurs naturally, so far as we know at present, only as free

or combined cysteine, *S*-cysteinossuccinic acid should be formed by hydrolysis of enzymes or coenzymes after their reaction with maleic acid. This view has been shown to be correct for *S*-glutathionossuccinic acid. Hydrolysis of this compound gave *S*-cysteinossuccinic acid. The product was partially racemized.

EXPERIMENTAL

Crystallization of S-cysteinossuccinic acid from methanol

(1) 0.4 g. amorphous condensation product of *l*-cysteine and maleic acid [Morgan & Friedmann, 1938, 1] was dissolved in hot methanol. On cooling the solution slowly deposited white crystals. After removal of the supernatant fluid the crystals are washed with methanol and dried *in vacuo* at 45° over P₂O₅. (Found: C, 35.1; H, 4.6; N, 5.6; S, 13.1%. C₇H₁₁O₆NS requires C, 35.5; H, 4.6; N, 5.9; S, 13.5%.) $[\alpha]_D^{20} = -29.75^\circ$, -29.84° ($c = 2.236$ in water).

(2) 0.4 g. amorphous *S*-cysteinossuccinic acid was dissolved in approximately 30 ml. of hot methanol. Next day the solution was slowly taken down to 4 ml. under slightly reduced pressure. 0.347 g. of white hedgehog crystals separated which were washed with methanol. Yield 86.75%. $[\alpha]_D^{20} = -27.58^\circ$ ($c = 2.357$ in water).

S-Cysteinossuccinic acid commences to shrink and soften at 102° and melts at 134–135° (decomp.). It is hygroscopic and the m.p. had to be taken after drying the substance in the melting tubes for several days *in vacuo* over P₂O₅. The substance is easily soluble in cold water, slightly soluble in cold, better in hot methanol. In hot glacial acetic acid it dissolves slowly and sparingly, apparently with decomposition which turns the solution yellow. It is not soluble in the other usual organic solvents.

Effect of glacial acetic acid on crystalline, laevorotatory S-cysteinossuccinic acid

(1) 0.1 g. crystalline *S*-cysteinossuccinic acid ($[\alpha]_D = -29.8^\circ$) was dissolved in boiling glacial acetic acid. On cooling 0.0745 g. of crystals deposited quickly as a mixture of forms mostly needles with some rosettes $[\alpha]_D^{20} = -5.12^\circ$ ($c = 2.246$ in water).

Another sample prepared in the same way was analysed after drying *in vacuo* over P₂O₅ and KOH at 40°. (Found: C, 35.9; H, 4.8; N, 5.7; S, 12.8%. C₇H₁₁O₆NS requires C, 35.5; H, 4.6; N, 5.9; S 13.5%.)

(2) 0.1 g. substance ($[\alpha]_D = -29.8^\circ$) was dissolved in boiling glacial acetic acid and allowed to crystallize overnight. The crystals were redissolved in the mother liquor by boiling. On cooling and standing overnight 0.0430 g. needles of uniform appearance were obtained. The mother liquor was deep yellow and gave on concentration a gummy residue. m.p. of the needles was 134–135° (decomp.) after softening at 125°. $[\alpha]_D$ in water was zero.

The substance used for polarimetry was recovered and analysed after drying as above. (Found: C, 35.5; H, 5.0%. C₇H₁₁O₆NS requires C, 35.5; H, 4.6%.)

S-Cysteinossuccinic acid obtained by hydrolysis of S-glutathionossuccinic acid

S-Glutathionossuccinic acid [Morgan & Friedmann, 1938, 1] was hydrolysed by Hopkins's [1929] method used for the hydrolysis of glutathione.

0.5 g. substance was boiled with 25 ml. of 25% H₂SO₄ for 16 hr. The solution, diluted to 800 ml., was precipitated with HgSO₄ avoiding excess. The Hg precipitate separated slowly. After 2 days it was collected and decomposed with H₂S. H₂SO₄ was removed from the filtrate quantitatively with Ba(OH)₂, the solution transferred to a crystallizing dish and evaporated *in vacuo* over

H_2SO_4 . Yield 0.21 g. Recrystallized from hot methanol, 0.090 g. hedgehog crystals were obtained. m.p. 134–135° after softening at 102°. For analysis the substance was dried *in vacuo* over P_2O_5 at 45°. (Found: C, 35.2; H, 4.8; N, 5.6%. $\text{C}_7\text{H}_{11}\text{O}_6\text{NS}$ requires C, 35.5; H, 4.6; N, 5.9%.) $[\alpha]_D^{25} - 6.43^\circ$ ($c = 1.786$ in water).

The properties of the isolated substance were identical with those of *S*-cysteinossuccinic acid.

SUMMARY

S-Cysteinossuccinic acid crystallizes from methanol in hedgehog crystals, softening at 102° and melting at 134–135° (decomp.).

On treatment with hot glacial acetic acid racemization occurs, apparently accompanied by partial decomposition.

S-Cysteinossuccinic acid is formed by hydrolysis of *S*-glutathionossuccinic acid.

The significance of *S*-cysteinossuccinic acid for the interpretation of the maleic acid reaction in enzyme work is pointed out.

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