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ON THE REACTION OF NINHYDRIN WITH CYSTEINE AND ITS ANALOGUES: A REVISION[a]

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Abstract—Chemical and spectroscopic evidence leads to the conclusion that the compound described in the literature as 2,3,9,9a-tetrahydro-9a-hydroxy-9-oxo-indeno[2.1]-*p*-thiazine-3-carboxylic acid (**1**), which is formed in the reaction of ninhydrin with L-cysteine, has in fact the isomeric spirane structure (**4**). Cysteine analogues, such as 3-mercapto-valine and 2-aminoethanethiol, react similarly to give the corresponding condensation products (**7** and **8**).

All α-aminoacids, with exception of cysteine and related compounds, react with ninhydrin to give diketohydrinylidenediketohydrindamine (also called Ruhemann's purple) in nearly stoichiometric amounts.[2,3] An explanation for the anomalous behaviour of cysteine was first offered by Kuhn and Hammer[4] in terms of preferential formation of a colourless condensation product, which was formulated as **1** although there was no supporting chemical evidence. As a part of a more recent study on the kinetics of the ninhydrin reaction, Friedman and Sigel[5] have substantiated the thiazine structure **1** by means of a PMR spectrum of the product (in CF_3COOH) showing, in addition to a 4 H signal at δ 8·18 for the aromatic protons, a triplet at δ 5·75 (1 H, J5·7 Hz) and a doublet at δ 4·13 (2 H, J5·7 Hz), assigned to the methine and methylene protons in **1**, respectively. Based also on the reactivity of some model compounds, they concluded that the anomalous behaviour of cysteine and its analogues was due to the more pronounced nucleophilic character of the thiol group compared to the amino group, favouring the formation of intermediates of the type **2** rather than **3**; only the latter can proceed to give the Ruhemann's purple, whereas the former ring-closes to the thiazine derivative **1**.[5]

A close examination of the data reported by previous workers suggested that an alternative structure for the condensation product of cysteine with ninhydrin, namely **4**, would not only accommodate the chemical and physical data but would also be mechanistically more plausible as one would expect the proposed intermediate **2**, as well as **3**, to cyclize preferentially at the more reactive 2-position of the indane skeleton. Accordingly, we have repeated the

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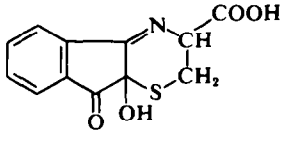

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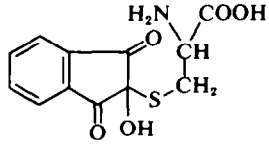

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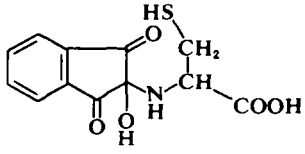

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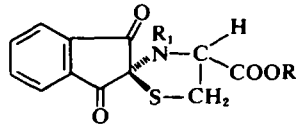


4: R = R_1 = H
5: R = Me; R_1 = H
6: R = R_1 = Me

reaction of ninhydrin with L-cysteine hydrochloride exactly as originally described by Kuhn and Hammer and have obtained the reported condensation product $C_{12}H_9NO_4S$, m.p. 167–168° (dec), $[\alpha]_D$-152° (in EtOH). However, examination of its spectroscopic properties did not allow a clear-cut choice between the two isomeric structures **1** and **4**, although the close similarity of the UV spectrum with that of ninhydrin favoured the spirane **4** rather than the thiazine **1**.

To secure structure **4** for the condensation product, it was then esterified with CH_2N_2 to give the corresponding methyl ester **5**, $C_{13}H_{11}NO_4S$, m.p. 82–84° (dec). Subsequent treatment of **5** with methyl iodide in anhydrous DMF, followed by preparative TLC of the reaction mixture on silica, afforded a Me derivative, $C_{14}H_{13}NO_4S$, m.p. 128–130° (dec), $[\alpha]_D$-106·5° (in CCl_4), the PMR spectrum of which was in agreement with the spirane structure **6**. In addition to the signals arising from the aromatic protons δ 7·90 and the carbomethoxyl group δ 3·79, the spectrum, taken in $CDCl_3$, exhibited a triplet (1 H) at δ 4·42 and a doublet (2 H) at δ 3·55 (J 6·6 Hz) for the methine and methylene protons of the thiazolidine ring, and a sharp singlet (3 H) at δ 2·47, shifted to δ 2·92 on addition of CF_3COOH, attributable to the N-Me group in **6**.

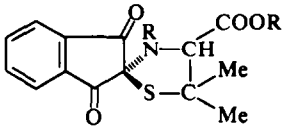
As expected, cysteine analogues, like 3-mercapto-valine (penicillamine) and 2-aminoethanethiol (cysteamine), reacted with ninhydrin similarly giving the corresponding condensation products, characterized as **7** and **8**, respectively. The former structural assignment was deduced from the sequential treatment of **7** with CH_2N_2 and methyl iodide leading to the N-methylderivative **9**, $C_{16}H_{17}NO_4S$, m.p. 108–110°.

Due to the difficulty in isolating the N-Me derivative of the condensation product of ninhydrin with cysteamine, evidence indicating the spirane structure **8** was provided by reduction with $NaBH_4$. This reaction afforded a crystalline compound, $C_{11}H_{13}NO_2S$, with an ill-defined m.p., showing in the PMR spectrum (DMSO-d_6) a pair of doublets (2 H each, J 7·3 Hz) at δ 4·83 and 5·72, attributable to two CHOH groups in **10**; by D-exchange the signal at δ 5·72 was removed while the doublet at δ 4·83 collapsed to a sharp singlet. However, analytical TLC on silica gel (eluent EtOAc) revealed that the product was in fact a mixture of two of the possible diastereoisomers, corresponding to the gross structure **10**. Attempts to isolate the isomeric diols by preparative TLC were unsuccessful owing to their poor solubility in the suitable solvent for elution (EtOAc). However, they could be separated after exhaustive acetylation of the mixture which afforded the corresponding triacetates, recognized as **11** and **12** on the basis of PMR spectroscopy.

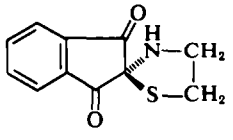
The relative stereochemistry of the two acetates followed from the difference in chemical shifts of the 2 H singlet corresponding to the equivalent *cis*-protons on the carbons bearing the acetoxyl groups. These protons in **12** resonated at a "normal" δ-value 6·46, while in **11** they were shifted lowfield to δ 7·09 by the effect of the neighboring *cis*-oriented N-acetyl group.

EXPERIMENTAL

Microanalyses were carried out by E. Thommen, Department of Organic Chemistry, University of Basel, Switzerland. M.ps were determined for samples in capillaries or with a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Infracord 137 E, UV spectra with a Perkin-Elmer 402 spec-



7: R = H
9: R = Me



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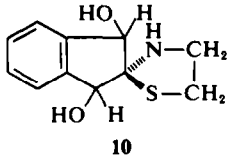

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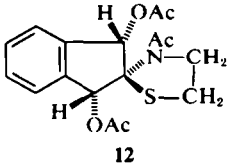

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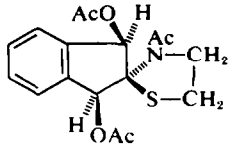

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trophotometer and PMR spectra with a Perkin-Elmer R-12 A spectrometer. Chemical shifts are expressed in δ values (ppm) downfield from TMS as internal reference. To some solutions deuterium oxide was added to check on exchangeable protons. Mass spectra were measured by the direct insertion technique with an AEI-MS 902 spectrometer (70 eV) with the lowest source temperature which produced a definite spectrum. Besides the molecular ion the most abundant ions in the mass spectrum (above *m/e* 100) are given with their relative intensities. Optical rotations were measured at room temperature with a Perkin-Elmer 141 polarimeter. Analytical and preparative TLC were carried out on Merck silica GF_{254} and all solvents used for the developments and for elution were redistilled. Proportions given for mixed solvents are by volume. Chromatograms were examined by UV irradiation at 254 nm or sprayed with 2% ceric sulfate in 2 N H_2SO_4 or with 2% $SbCl_3$ in EtOH.

1, 3-*Dione-4'-carboxy-spiro* [*indane-2,2'-thiazolidine*], **4**. Following the procedure described by Kuhn and Hammer, 3 mmoles of L-cysteine hydrochloride were allowed to react with 3 mmoles of ninhydrin in aqueous soln. The ppt which formed on cooling was collected by filtration and was recrystallized from EtOH to give **4** (51% yield), m.p. 167–168° (dec). (Found: C, 54·93; H, 3·60; N, 5·15; S, 12·37. $C_{12}H_9NO_4S$ requires: C, 54·76; H, 3·45; N, 5·32; S, 12·16%); *m/e* 263 (M^+, 100), 218 (51), 136 (47), 105 (29), and 104 (33%); λ_{max} (MeOH) 231, 246 (sh) nm (log ε 4·43, 4·01); ν_{max} (Nujol) 3266, 1745, and 1718 cm^{-1}. The PMR spectrum in CF_3COOH was identical with that reported by Friedman and Sigel.

1,3-*Dione-4'-carbomethoxy-spiro* [*indane-2,2'-thiazolidine*], **5**. To a soln of **4** (100 mg) in MeOH (15 ml) an excess ethereal CH_2N_2 was added. The mixture was left at room temp overnight and, after removal of the solvents, the residue was crystallized from EtOAc to give 90 mg of **5** as pale yellow needles, m.p. 82–84° (dec), $[\alpha]_D$ − 129·6° (in EtOH); *m/e* 277 (M^+, 100), 218 (69), 136 (42), 105 (25), and 104 (36%); λ_{max} (in EtOH) 229, 246 (sh) nm (log ε 4·46, 4·11); ν_{max} ($CHCl_3$) 3300, 1750, 1722 cm^{-1}; δ (CF_3COOH) 4·06 (2 H, d, J 6·0 Hz, CH_2), 4·12 (3 H, s, OMe), 5·63 (1 H, t, J 6·0 Hz, CH), 8·19 (4 H, s, aromatic).

1,3-*Dione-4'-carbomethoxy-3'-methyl-spiro* [*indane-2,2'-thiazolidine*], **6**. To a soln of **5** (320 mg) in anhydrous DMF (2 ml) MeI (0·2 ml) was added. After standing at room temp for 70 hr, the mixture was heated on a steam bath for 15 min, diluted with chloroform, washed with $NaHCO_3$ aq and evaporated. Preparative TLC of the residue on silica (C_6H_6–Et_2O) (85/15) afforded 45 mg of **6**, yellow prisms from CCl_4, m.p. 128–130°, $[\alpha]_D$ − 106·5° (in CCl_4), *m/e* 291 (M^+, 20), 232 (27), 136 (100), 105 (17), and 104 (33%); λ_{max} (MeOH) 235, 250 (sh), 287 nm (log ε 4·76, 4·44, 3·68); ν_{max} (CCl_4) 1750, 1720 cm^{-1}.

1,3-*Dione-4'-carboxy-5',5'-dimethyl-spiro* [*indane-2,2'-thiazolidine*], **7**. To a soln of ninhydrin (900 mg) in water (200 ml) was added dropwise with stirring an aqueous soln of D,L- penicillamine (740 mg) under cooling. After 30 min, the separated crystals were collected by filtration and recrystallized from EtOAc to give 800 mg of **7** (55% yield) as pale yellow prisms, m.p. 110–111° (dec). (Found: C, 57·87; H, 4·18; N, 4·96; S, 10·57; $C_{14}H_{13}NO_4S$ requires: C, 57·73; H, 4·50; N, 4·82; S, 10·98%); *m/e* 291 (M^+, 100), 246 (20), 231 (10), 230 (15), 136 (38), 105 (12), and 104 (28%); λ_{max} (MeOH) 232, 246 (sh) nm (log ε 4·48, 4·18); ν_{max} ($CHCl_3$) 3500–2400 br, 1760, 1740 (sh), 1722 cm^{-1}; δ (CF_3COOH) 1·82 (3 H, s, Me), 1·92 (3 H, s, Me), 5·34 (1 H, s, CH), 8·13 (4 H, s, aromatic).

1,3-*Dione-4'-carbomethoxy-3',5',5'-trimethyl-spiro* [*indane-2,2'-thiazolidine*], **9**. Methylation of **7** (325 mg) with excess ethereal CH_2N_2 gave the corresponding Me ester (270 mg), yellow needles from MeOH, m.p. 135–136°, *m/e* 305 (M^+, 100), 246 (30), 231 (12), 230 (15), 136 (15), 105 (6), and 104 (12%); λ_{max} (MeOH) 228, 248 (sh) nm (log ε 4·66, 4·10); ν_{max} ($CHCl_3$) 3275, 1740, 1719 cm^{-1}; δ ($CDCl_3$) 1·39 (3 H, s, Me), 1·71 (3 H, s, Me), 3·81 (3 H, s, OMe), 4·12 (1 H, br, NH, removed by D-exchange), 4·45 (1 H, br, CH, sharpened by D-exchange), 7·95 (4 H, m, aromatic). A soln of the ester (215 mg) in anhydrous DMF (3 ml) was treated with MeI (0·2 ml) as described for the methylation of the analogue **5**. The residue obtained from the chloroform extract was fractionated by preparative TLC with C_6H_6–Et_2O (70/30) to give 40 mg of **9** (R_f 0·54), yellow prisms from EtOH, m.p. 108–110°, *m/e* 319 (M^+, 93), 305 (20), 260 (73), 245 (23), 136 (100), 105 (10), and 104 (27%); λ_{max} (EtOH) 229, 248 (sh) nm (log ε 4·68, 4·11); ν_{max} ($CHCl_3$) 1750, 1710 cm^{-1}; δ ($CDCl_3$) 1·58 (3 H, s, Me), 1·64 (3 H, s, Me), 2·42 (3 H, s, N-Me), 3·78 (3 H, s, OMe), 4·48 (1 H, s, CH), 7·92 (4 H, m, aromatic).

1,3-*Dione-spiro* [*indane-2,2'-thiazolidine*], **8**. To a soln of ninhydrin (3·6 g) in water (100 ml) 2-aminoethanethiol hydrochloride (2·28 g) was added. The ppt which formed on cooling was collected by filtration, dried over P_2O_5 *in vacuo*, and extracted with chloroform. The resulting soln was evaporated to dryness and the residue was crystallized from EtOAc to give 1·5 g of **8**, yellow prisms, m.p. 150–151° (dec). (Found: C, 60·07; H, 4·21; N, 5·95; S, 14·12. $C_{11}H_9NO_2S$ requires: C, 60·27; H, 4·14; N, 6·39; S, 14·60%); *m/e* 219 (M^+, 100), 176 (27), 136 (98), 105 (61), and 104 (77%); λ_{max} (MeOH) 228, 246 (sh) nm (log ε 4·62, 4·08); ν_{max} (Nujol) 3440, 1739, 1712 cm^{-1}; δ ($CDCl_3$) 2·62 (1 H, br, NH, removed by D-exchange), 3·23 (2 H, t, J 6·0 Hz, N-CH_2), 3·80 (2 H, t, J 6·0 Hz, S-CH_2), 7·90 (4 H, m, aromatic).

1,3-*Dihydroxy-spiro* [*indane-2,2'-thiazolidine*], **10**. To a soln of **8** (500 mg) in MeOH (100 ml) 250 mg of $NaBH_4$ was added. After standing at room temp for 30 min, the mixture was concentrated to a small volume *in vacuo*, diluted with water, and then extracted with EtOAc. Evaporation of the combined extracts gave a crystalline residue which was recrystallized twice from MeOH to yield 190 mg of **10**, showing two distinct spots on analytical TLC (R_f 0·56 and 0·31 in EtOAc), m.p. 155–162°. (Found: C, 59·82; H, 5·41; N, 6·08; S, 14·70. $C_{11}H_{13}NO_2S$ requires: C, 59·18; H, 5·87; N, 6·28; S, 14·31%); *m/e* 223 (M^+, 5), 205 (61), 188 (22), 177 (52), 149 (100), 148 (33), and 105 (16%); λ_{max} (MeOH) 215 nm (log ε 3·98); ν_{max} (Nujol) 3560–3130 br cm^{-1}.

Acetylation of 1,3-*dihydroxy-spiro* [*indane-2, 2'-thiazolidine*], **10**

Isolation of stereoisomeric triacetates **11** *and* **12**. A mixture of **10** (200 mg), Ac_2O (5 ml) and pyridine (5 ml) was allowed to stand at room temp 76 hr. The product obtained after usual work up was fractionated by column chromatography on silica (eluent Et_2O) to give pure **11** (more polar, 40 mg) and pure **12** (less polar, 55 mg). Compound **11** formed prisms from EtOAc, m.p. 178–179°, *m/e* 349 (M^+, 6), 289 (98), 264 (24), 247 (62), 205 (20), 204 (41), 188 (100), 177 (18), 176 (26), and 149 (22%); λ_{max} (EtOH) 212, 271 nm(log ε 4·05, 3·76); ν_{max} ($CHCl_3$) 1734, 1647 cm^{-1}; δ ($CDCl_3$) 2·15 (3 H, s, N-Ac), 2·18 (6 H, s, O-Ac), 2·82 (2 H, t, J 6·0 Hz, N-CH_2), 3·81 (2 H, t, J 6·0 Hz, S-CH_2), 7·09 (2 H, s, CH-OAc), 7·33 (4 H, m, aromatic). Compound **12**, prisms from EtOAc, m.p. 157–158°, *m/e* 349 (M^+, 3), 289 (50), 264 (15), 247 (35), 205 (16), 204 (45), 188 (100), 177

(15), 176 (20), and 149 (25%); λ_{max} (EtOH) 212, 270 nm (log ϵ 4·09, 3·78); ν_{max} ($CHCl_3$) 1738, 1667 cm^{-1}; δ ($CDCl_3$) 1·94 (3 H, s, N-Ac), 2·16 (6 H, s, O-Ac), 3·04 (2 H, t, J 6·0 Hz, N-CH_2), 3·98 (2 H, t, J 6·0 Hz, S-CH_2), 6·46 (2 H, s, CH-OAc), 7·33 (4 H, m, aromatic).

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