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The Structures of Isatylidene-3-mercaptoacetic Acid and Its Related Compounds[1]

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It was proved that an alkaline-hydrolysis product of isatylidene-3-rhodanine (I) was not 1,2-dihydro-3-mercapto-2-oxocinchoninic acid (III) reported by Gränacher, but isatylidene-3-mercaptoacetic acid(II). On the basis of this assignment, some structures of its derivatives incorrectly reported were revised. Moreover, the configurations of II and its derivatives are discussed.

During the course of a research for biologically active cinchoninic acid derivatives, it became necessary to re-examine the structure of 1,2-dihydro-3-mercapto-2-oxocinchoninic acid (III) which was assigned[3,4] to the alkaline-hydrolysis product (hereinafter refered to as compound A) of isatylidene-3-rhodanine (I). This paper deals with some findings concerning structures and configurations of compound A and its related compounds, including the correct assignment of compound A to isatylidene-3-mercaptoacetic acid (II).[5]

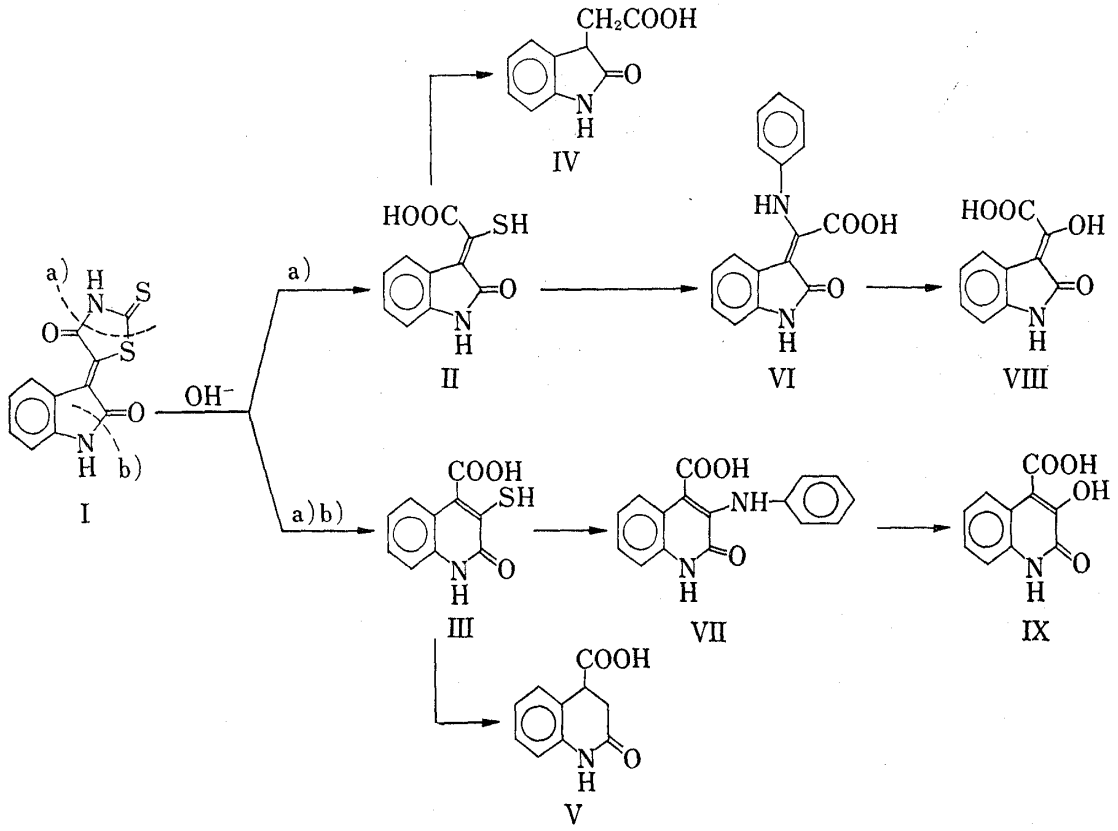


Chart 1

1) Presented at the 22nd Meeting of Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, Nov. 1972.
2) Location: *Enoki 33-94, 564 Suita City, Osaka.*
3) Ch. Gränacher and Ch. Kouniniotis, *Helv. Chim. Acta.*, **11**, 1241 (1928).
4) J.A. Aeschlimann, *J. Chem. Soc.*, **1926**, 2902.
5) Ch. Gränacher and A. Mahal, *Helv. Chim. Acta.*, **6**, 467 (1923).

Cränacher and Mahal,[5] in 1923, reported the preparation of II by alkaline hydrolysis of I and prepared so-called "Gränacher's oxindoleacetic acid" (IV) by reduction of II. They also synthesized some isatylidene derivatives from II including isatylidene-3-anilinoacetic acid (VI) which was converted by hydrolysis to isatylidene-3-hydroxyacetic acid (VIII). Aeschlimann,[4] however, proved that the structure of "Gränacher's oxindoleacetic acid" was not actually oxindole-3-acetic acid (IV), but 2-oxo-1,2,3,4-tetrahydrocinchoninic acid (V), and therefore, proposed the formula III for the structure of its precursor, compound A. Afterward, Gränacher[3] confirmed Aeschlimann's findings and then revised all the structural formulas of isatylidene derivatives such as II, VI and VIII to the formulas of the corresponding cinchoninic acid derivatives such as III, VII and IX (see Chart 1). Ever since, compound A has been considered to be the structure III.

On the other hand, both compounds, VIII and IX,[6] were prepared by Wislicenus[7] using alternative methods in 1924 before Gränacher's revision. The melting point and color of Gränacher's revised compound (IX) turned out to be in good agreement with those of Wislicenus's compound (VIII). This fact suggests that the structure of compound A is isatylidene form, contrary to Gränacher's revision.

In some efforts to elucidate the structure of compound A, the reaction of compound A with methyl isothiocyanate gave a deep-purple crystalline product,[8] which was identical with isatylidene-3-N-methylrhodanine (X) obtained by condensation of isatin with N-methylrhodanine (see Chart 2). This finding proved that the correct structure of compound A was

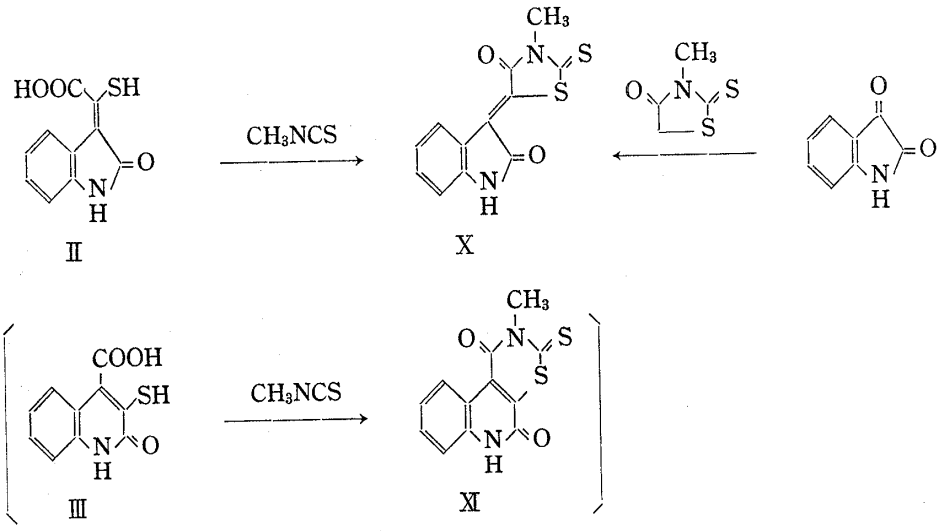


Chart 2

isatylidene-3-mercaptoacetic acid (II) as formulated in Gränacher's first paper.[5] Therefore, all the structural assignments for derivatives[3,9] of compound A based on the formula III should be re-examined except the compound V.

As reported by Gränacher, it was difficult to purify the compound II. The difficulty may be due to the co-existence of some tautomers of II and the instability of II in a solution which will be discussed later. According to Gränacher, toluene was the only useful solvent for

6) B. Eistert and H. Selzer, *Chem. Ber.*, **96**, 1234 (1963).
7) W. Wislicenus and H. Bubeck, *Ann.*, **436**, 113 (1924).
8) This compound is considered to be the same compound as the thiazino quinolone derivative (XI) which was prepared by the reaction of III with methyl isothiocyanate by Kretov, *et al.*[9b].
9) *a*) R.V. Jones, *J. Am. Chem. Soc.*, **64**, 1672 (1942); *b*) A.E. Kretov, A.P. Momsenko, A.S. Bespalyi and Yu. A. Levin, *Khim. Geterotsikl. Soedin.*, **1968**, 99 [*Chem. Abstr.*, **69**, 86928*t* (1968)]; *c*) *Idem*, *ibid.*, **1973**, 641 [*Chem. Abstr.*, **79**, 42431*c* (1973)]; *d*) A.E. Kretov, A.P. Momsenko and Yu.A. Levin, *ibid.*, **1973**, 644 [*Chem. Abstr.*, **79**, 42434*f* (1973)].

recrystallization of II and the use of other solvents only gave an oily substance. However, recrystallization from toluene did not give analytically pure crystals. Our attempt to use *tert*-butanol–hexane was successful.

The nuclear magnetic resonance (NMR) spectrum of II in dimethyl sulfoxide-d_6 shows three independent peaks (presumably N–H[10]) at 10.67, 10.86 and 10.94 ppm which suggest the co-existence of three tautomers (see Fig. 1). Three tautomers are speculated to be a *trans*[11] ene-thiol form (IIa), a *cis* ene-thiol form (IIb) and a thione form (IIc) (see Chart 3). In Fig. 1, the apparent doublet at 7.80 ppm corresponds to about one third hydrogens and may be assigned to the aromatic hydrogen at C-4 of the *trans* ene-thiol form (IIa). In a more simplified system such as α-thioacyllactone (see Chart 4) similar to the above system, Duus, *et al.*[12a] proved the existence of tautomerism between two ene-thiol forms by NMR spectroscopy. Duus, *et al.*,[12b] likewise confirmed that some thioacetothiolesters could exist in three different forms, namely two ene-thiol forms and a thioketo form (see Chart 4) by NMR and

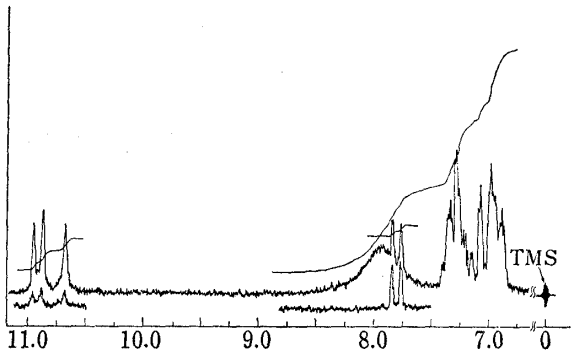
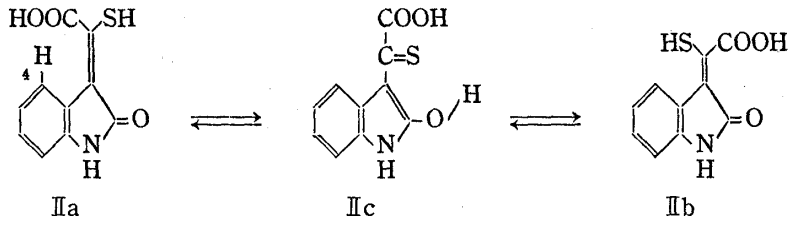
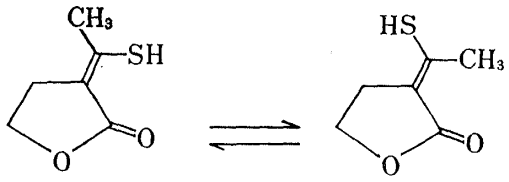


Fig. 1. NMR Spectrum of Isatylidene-3-mercaptoacetic Acid(II) in DMSO-d_6 at 100 MHz

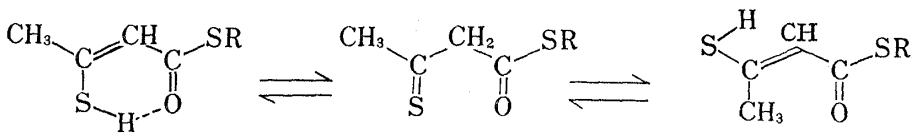


IIa IIc IIb

Chart 3



α-thioacyllactone



thioacetothiolester

Chart 4

10) This presumption is supported from NMR spectra of other analogs listed in Table I.

11) The usage of *trans* and *cis* applied to isatylidene-3-acetic acid analogs in this paper is based on considering these compounds as analogs of fumaric and maleic acids.

12) *a*) F. Duus, E.B. Pedersen and S.-O. Lawesson, *Tetrahedron*, **25**, 5703 (1969); *b*) F. Duus, P. Jakobsen and S.-O. Lawesson, *ibid.*, **24**, 5323 (1968).

infrared (IR) studies. On methylation with methyl iodide in a cooled alkaline solution, II gave predominantly *trans*-isatylidene-3-methylmercaptoacetic acid (XIIa) and a small amount of the mixture of *trans* and *cis* compounds, XIIa and XIIb, which was confirmed by NMR spectroscopy and thin-layer chromatography (TLC) (see Experimental section). When heated in 20% NaOH, XIIa gave the *cis* isomer (XIIb) in a good yield. The structure of XIIb was presumed on the basis of its NMR spectrum (see Table I) and further confirmed by the following chemical means; both compounds (XIIa and XIIb), on reaction with aniline, gave the same product, VI (see Chart 5). On the other hand, it is known that some isatylidene-3-

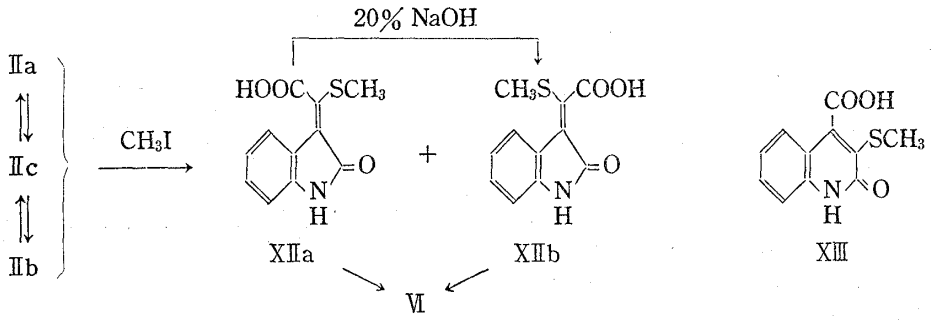


Chart 5

acetic acid analogs[13] are converted to the corresponding cinchoninic acid analogs. Some attempts to convert II and XIIa to the corresponding cinchoninic acids (III and XIII) with the S-atom at C-3 were made. On treatment of XIIa with 20% NaOH, there occurred no ring transformation to 1,2-dihydro-2-oxo-3-methylmercaptocinchoninic acid (XIII),[14] but isomerization to the *cis* isomer (XIIb) as described above. When heated in 6% HCl, XIIa mainly remained unchanged and partly decomposed. On reaction of XIIa with 48% HBr in AcOH, there was only obtained oxindole in a low yield. Thus, the formation of XIII from XIIa was

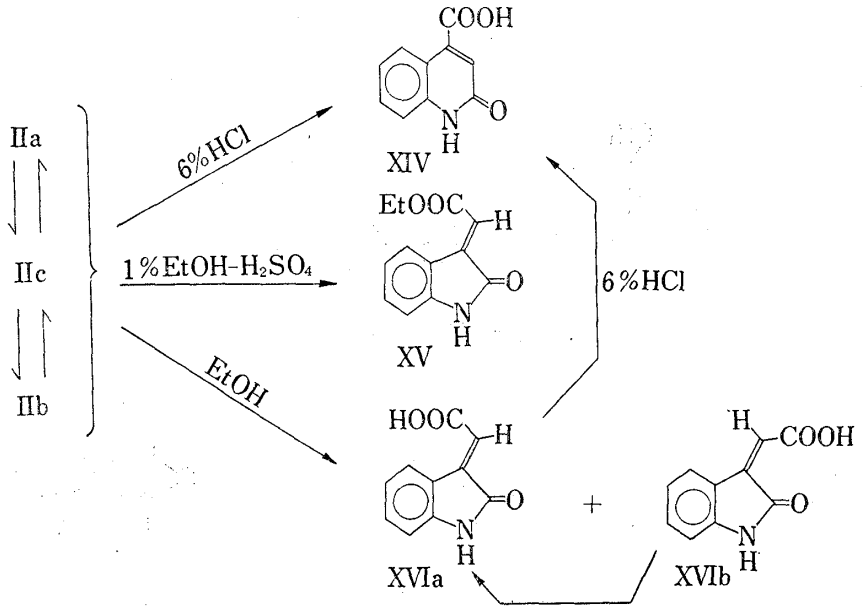
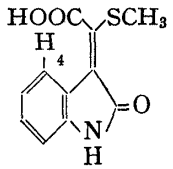
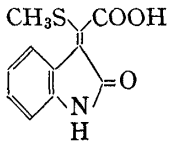
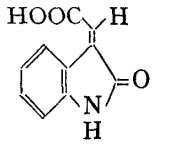
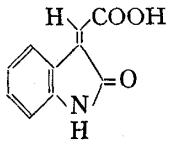
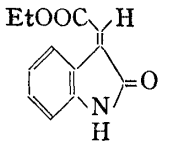
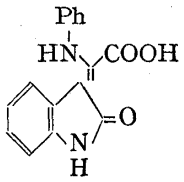
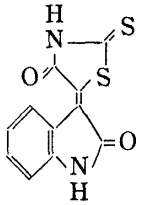
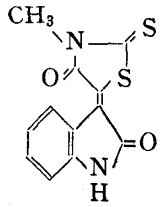


Chart 6

13) *a*) H.C. Van der Plas, "Ring Transformations of Heterocycles," Vol. 1, 218, London and New York, 1973, and references cited therein; *b*) P.L. Julian, H.C. Printy, R. Ketcham and R. Doone, *J. Am. Chem. Soc.*, **75**, 5305 (1953); *c*) G. Jones and W.J. Rae, *Tetrahedron*, **22**, 3021 (1966).

14) This compound reported by Jones[9a] is considered to be the same compound as XIIa and must be here revised to the formula XIIa.

TABLE I. NMR Spectral Data[a] of Isatylidene Derivatives

Compound		H-C-4[b] (or aromatic H)	-CONH-	-COOH	Other feature
XIIa		7.70	10.60	13.83 (br)	2.55 (3H, s, S-CH_3)
XIIb		6.80—7.45 (4H, m)	10.72	12.42 (br)	2.43 (3H, s, S-CH_3)
XVIa[c]		8.32	10.72	13.30 (br)	6.63 (1H, s, vinyl H)
XVIb[c]		7.72	11.13	13.85 (br)	7.19 (1H, s, vinyl H)
XV		8.39	10.80		6.67 (1H, s, vinyl H)
VI		6.80—7.53 (9H, m)	10.87 (or 11.62)	10.92 (br)	11.62 (or 10.87) (1H, br, -NHPh)
I		8.80	11.14 (or 13.90)		13.90 (or 11.14) (1H, br, -CONHCS-)
X[d]		9.19	undetectable		3.45 (3H, s, N-CH_3)

a) Recorded at 60 MHz on a Varian A-60 spectrometer unless otherwise noted. Chemical shifts are expressed in ppm on the δ scale. All compounds were measured in DMSO-d_6 unless otherwise noted. Abbreviation used s=singlet, m=multiplet, br=broad.
b) This proton exhibited a doublet (J=~7 cps) with further splitting.
c) recorded at 100 MHz on a Varian HA-100D spectrometer
d) dissolved in pyridine-d_5
Ph=phenyl

not observed. When heated in 6% HCl, however, II gave 1,2-dihydro-2-oxocinchoninic acid (XIV). Such ring transformation with desulfurization was of interest and further investigated. When heated under reflux in ethanolic 1% sulfuric acid, II afforded ethyl isatylidene-3-acetate (XV)[13b] with desulfurization. II in EtOH, on merely refluxing or on standing for a few days, gave predominantly unknown *trans*-isatylidene-3-acetic acid (XVIa)[15] and a small amount of the *cis* isomer (XVIb), with precipitation of sulfur (see Chart 6). These two acids (XVIa and XVIb) were isolated by preparative TLC and their configurations were confirmed by means of their NMR spectra. It was found that such desulfurization readily occurred also in an ordinary organic solvent such as acetone or benzene, by TLC and NMR studies. Such instability of II seems to make purification more difficult. On treatment with 6% HCl, XVIa was easily transformed to XIV. It was also made clear that the *cis* acid (XVIb) was converted to the *trans* acid (XVIa) by NMR studies, in which a solution of XVIb in DMSO-d_6 containing D_2O, on standing overnight, showed the spectrum of XVIa. From the result mentioned above, the desulfurization from II to XIV turned out to take place before ring transformation.

NMR spectra provided useful informations concerning configurations of isatylidene derivatives. In an isatylidene derivative with a carbonyl group at β position to C-3, it is known[13c,16] that the aromatic ring proton at C-4 is deshielded by the carbonyl group trans to the ring lactam group. Therefore, configurations of isatylidene derivatives described above were determined on the basis of the chemical shifts of C-4 protons tabulated in Table I. The especially strong downfield-shift of the C-4 proton in each case of I and X may be due to the proximity of the carbonyl group fixed by the ring formation. The anilino acid (VI) may be assigned to the *cis* configuration because of no downfield shift of the C-4 proton.

Experimental[17]

Isatylidene-3-mercaptoacetic Acid (II)——According to the procedure of Gränacher[3,5], II was prepared by refluxing isatylidene-3-rhodanine (I) in 10% NaOH for 30 min. The crude product obtained was repeatedly recrystallized from a mixture of *tert*-butanol and hexane, yielding orange needles of II, mp 169—172° (decomp.) (*lit.*[3] mp 165—167° (decomp.)). Mass Spectrum *m/e*: 221 (M^+), 177 (M^+-CO_2), 175 (base). UV λ_{max}^{MeOH} nm (log ε): 253 (4.03), 274 (3.82), 378 (4.05). IR ν_{max}^{KBr} cm^{-1}: 3220 (br), 2475, 2430 (sh), 1700 (sh), 1690, 1610 (sh), 1570, 1545. *Anal.* Calcd for $C_{10}H_7O_3NS$: C, 54.29; H, 3.19; N 6.33; S, 14.49. Found: C, 54.56; H, 3.36; N, 6.21; S, 14.33.

Isatylidene-3-N-methylrhodanine (X)——a) From Isatin: To a hot solution of isatin (5.0 g) and AcOH (30 ml) was added N-methylrhodanine (5.0 g) and then the mixture was refluxed on a flame for 2 hr. The mixture was cooled and the precipitates were collected by filtration, washed with AcOH, H_2O and EtOH in turn and dried in vacuo, yielding 3.0 g of X. Recrystallization from dioxane gave deep-purple needles, mp 308—310°. Mass Spectrum *m/e*: 276 (M^+), 175 (base). IR ν_{max}^{KBr} cm^{-1}: 1680, 1610. *Anal.* Calcd for $C_{12}H_8O_2N_2S_2$: C, 52.16; H, 2.92; N, 10.14; S, 23.21. Found: C, 52.23; H, 3.09; N, 10.03; S, 23.19.

b) From II: A solution of II (11.0 g) and methyl isothiocyanate (7.0 g) in dioxane (40 ml) was heated on a boiling-water bath for 3.5 hr. After cooling, deep-purple needles precipitated were collected by filtration and recrystallized from dioxane, yielding X (3.0 g), mp and mixed mp 308—310°, identified by comparison of its IR and Mass spectra with those of a sample prepared from isatin.

Methylation of II with Methyl Iodide——To a stirred cold solution of II (1.0 g) in 10% NaOH (5 ml) was added dropwise methyl iodide (1.5 g). After the solution had been stirred for 3 hr in an ice bath and then for 1 hr at room temperature, yellow sodium salts of XIIa precipitated were collected by filtration and dissolved in hot water. The resulting solution was acidified with *dil.* HCl to precipitate yellow leaflets which were collected by filtration. The alkaline filtrate was also acidified with *dil.* HCl to precipitate yellow leaflets which were collected by filtration. The combined leaflets amounted to 0.6 g of *trans*-isatylidene-3-methylmercaptoacetic acid(XIIa) which was recrystallized from aqueous EtOH to show mp 219—220° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 1720 (sh), 1705, 1655, 1620. UV λ_{max}^{MeOH} nm (ε): 253 (15000), 284 (6680), 332 (14700). *Anal.* Calcd. for $C_{11}H_9O_3NS$: C,

15) This compound has not yet been isolated and has been considered[4,13a] to be the extremely labile intermediate in the synthesis of 1,2-dihydro-2-oxocinchoninic acid (XIV) by the reaction of isatin with malonic acid.

16) R.L. Autrey and F.C. Tahk, *Tetrahedron*, **23**, 901 (1967).

17) All melting points were measured on a Yanagimoto Micro Melting Point Apparatus and are uncorrected. IR, UV, Mass and NMR spectra were taken on a Hitachi 215 spectrophotometer, a Hitachi EPS-2U spectrophotometer, a Hitachi RMU-6L spectrometer and a Varian A-60 (or HA-100D) spectrometer, respectively. Abbreviation used s=singlet, m=multiplet, br=broad and sh=shoulder.

56.16; H, 3.86; N, 5.95; S, 13.65. Found: C, 55.95; H, 3.68; N, 5.94; S 13.43. The combined acidic filtrate was concentrated *in vacuo* to a small amount of solution. After cooling, yellow leaflets precipitated were collected by filtration and recrystallized from a mixture of EtOH and hexane, yielding 20 mg of the mixture of XIIa and XIIb, mp 213—216°. TLC on silica gel (solvent: HCOOEt–HCOOH 20: 1) showed clearly two spots of XIIa and XIIb. Its NMR spectrum indicated that the ratio of XIIa to XIIb was about 3: 2. NMR (in DMSO-d_6) δ: 2.43 (s, S-CH_3, XIIb), 2.55 (s, S-CH_3, XIIa), 6.80—7.47 (m, aromatic, XIIa and XIIb), 7.70 (m, C-4 proton, XIIa), 8.6 (br, COOH, XIIa and XIIb), 10.60 (br, N-H, XIIa), 10.72 (br, N-H, XIIb). *Anal.* Calcd. for $C_{11}H_9O_3NS$ (XIIa and XIIb): C, 56.16; H, 3.86; N, 5.95; S, 13.63. Found: C, 56.05; H, 3.72; N, 6.15; S, 13.32.

***cis*-Isatylidene-3-methylmercaptoacetic Acid (XIIb)**——A solution of XIIa (0.5 g) in 20% NaOH was heated on a boiling-water bath for 40 min and then allowed to stand overnight at room temperature. The solution was acidified with dil. HCl to precipitate yellow leaflets. The precipitates were collected by filtration and recrystallized from a mixture of EtOH and hexane yielding yellow leaflets (0.3 g) of XIIb, mp 223—224° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 1700 (sh), 1680, 1615. UV λ_{max}^{MeOH} nm (ε): 277 (11500), 338 (13700). *Anal.* Calcd for $C_{11}H_9O_3NS$: C, 56.16; H, 3.86; N, 5.95; S, 13.63. Found: C, 56.14; H, 4.14; N, 5.84; S, 13.34.

***cis*-Isatylidene-3-anilinoacetic Acid (VI)**——a) From XIIa: A solution of XIIa (2.0 g) and aniline (3.2 g) in EtOH (50 ml) was refluxed for 4 hr. After cooling, yellow aniline salts of VI which precipitated were collected by filtration and dissolved in *dil.* NH_4OH. The resulting solution was neutralized with *dil.* HCl to precipitate yellow crystalline powders. The crude powders were collected by filtration and recrystallized from a mixture of EtOH and hexane, yielding yellow crystalline powders (1.5 g) of VI, mp 230—232° (*lit.*[5] mp 232°). IR ν_{max}^{KBr} cm^{-1}: 1705, 1610, 1585. UV λ_{max}^{MeOH} nm (ε): 276 (15800), 369 (25000). *Anal.* Calcd. for $C_{16}H_{12}O_3N_2$: C, 68.56; H, 4.32; N, 10.00. Found: C, 68.48; H, 4.29; N, 10.01.

b) From XIIb: According to the procedure similar to that used in the preparation of VI from XIIa described above, XIIb (0.3 g) was converted to VI in 18% yield. The product obtained was identified by mixed mp measurement and comparison of its IR and NMR spectra with those of a sample prepared from XIIa.

Oxindole from XIIa——A mixture of XIIa (1.0 g), 48% HBr (24 ml), AcOH (16 ml) and H_2O (8 ml) was heated under reflux for 1 hr. The mixture was poured into water and the aqueous solution extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with *dil.* NaOH and then *dil.* HCl and dried over anhydrous sodium sulfate. The solution was filtered and the filtrate concentrated *in vacuo*. The residue was recrystallized from a mixture of ether and hexane, yielding needles (0.1 g) of oxindole, mp 124—125°. The identity was confirmed by comparison of its IR spectrum with that of an authentic specimen.

1,2-Dihydro-2-oxocinchoninic Acid (XIV)——a) From II: A mixture of II (0.3 g) and 6% HCl (15 ml) was refluxed for 1 hr. The hot mixture was filtered in order to remove black insoluble materials. The resulting hot filtrate was cooled to afford yellow precipitates (0.13 g) of XIV. Recrystallization from EtOH gave an analytical sample, mp over 300° (*lit.*[4] mp 343°), which was identified by comparison of its IR spectrum with that of a specimen prepared according to the procedure of Aeschlimann.[4]

b) From XVIa: A mixture of XVI (35 mg) and 6% HCl (2 ml) was refluxed for 1 hr. After cooling, yellow precipitates were collected by filtration and recrystallized from EtOH, yielding XIV (24 mg), identified by comparison of its IR spectrum with that of a sample prepared from II.

Ethyl Isatylidene-3-acetate (XV)——A solution of II (1.0 g) in ethanolic 1% sulfuric acid (50 ml) was refluxed for 3 hr. After cooling, yellow long needles of sulfur which precipitated were filtered off and the filtrate was concentrated *in vacuo* and diluted with water. The precipitates were extracted with $CHCl_3$. The $CHCl_3$ solution was washed with *dil.* NaOH and then water, dried over anhydrous sodium sulfate and evaporated to dryness. The residual crystals were recrystallized from a mixture of benzene and hexane, yielding orange needles (0.45 g) of XV, mp 167—168° (*lit.*[13b] mp 169—170°). IR ν_{max}^{KBr} cm^{-1}: 1720 (sh), 1710, 1615. UV λ_{max}^{MeOH} nm (ε): 254 (21800), 316 (7300). *Anal.* Calcd. for $C_{12}H_{11}O_3N$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.35; H, 5.15; N, 6.41.

Desulfurization of II——A solution of II (3.0 g) in EtOH was refluxed for 1 hr. After cooling, the precipitates (0.3 g) of sulfur were filtered off. The filtrate was evaporated to dryness. To the residue was added AcOH (10 ml). Orange insoluble materials (0.9 g) were collected by filtration. On standing overnight, the filtrate afforded the additional precipitates (0.5 g). TLC of the product (1.4 g) thus obtained on silica gel (solvent system: $CHCl_3$–HCOOEt–HCOOH=5: 10: 1) showed two spots of *trans*- and *cis*-isatylidene-3-acetic acids (XVIa and XVIb). Of the mixture obtained, 170 mg was chromatographed on silica gel of preparative TLC with the same solvent system as used above, yielding XVIa (90 mg), XVIb (5 mg) and the mixture (50 mg). XVIa and XVIb were recrystallized from a mixture of iso-PrOH and hexane and showed mp 206—208° (decomp.) and mp 209—211° (decomp.), respectively. IR ν_{max}^{KBr} cm^{-1} (XVIa): 1720 (sh), 1690, 1620. UV λ_{max}^{MeOH} nm (ε) (XVIa): 253 (18900), 304 (5400). *Anal.* Calcd. for $C_{10}H_7O_3N$ (XVIa): C, 63.49; H, 3.73; N, 7.41. Found: C, 63.55; H, 4.01; N, 7.33. IR ν_{max}^{KBr} cm^{-1} (XVIb): 1700, 1605. UV λ_{max}^{MeOH} nm (ε) (XVIb): 255 (21500), 261 (20800), 304 (5400). *Anal.* Calcd for $C_{10}H_7O_3N$ (XVIb): C, 63.49; H, 3.73; N, 7.41. Found: C, 63.28; H, 3.63; N, 7.26.

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