those obtained from intravenous doses. The apparent inconsistency between this observation and some earlier reports may be explained by the relatively homogeneous patient population used in this study and the heterogeneous (at least regarding the degree of hepatic involvement) patient group (1) or the very small numbers of subjects (2, 13) used previously. Considerable variability in circulating levels of fluorouracil also was reported following intravenous doses to male and female patients (4).

While it is recognized that the clinical effectiveness of fluorouracil is a complex function of the anabolic metabolism of fluorouracil and the biochemistry of cell death and cell resistance, the present results indicate that oral fluorouracil in this patient population is absorbed with reproducible efficiency, resulting in no greater variation in circulating drug levels than that following intravenous doses.

This observation may have important implications in fluorouracil treatment of patients with breast cancer, particularly in view of the relationship that exists between the patient response rate and circulating levels of fluorouracil (15).

REFERENCES

(1) J. L. Cohen, L. E. Irwin, G. J. Marshall, H. Darvey, and J. R. Bateman, *Cancer Chemother. Rep.*, **58,** 723 (1974).

(2) E. R. Garrett, G. H. Hurst, and J. R. Green, *J. Pharm. Sci.*, **66,** 1422 (1977).

(3) W. E. MacMillan, W. H. Wolberg, and P. G. Welling, *Cancer Res.*, **38,** 3479 (1978).

(4) D. S. Sitar, D. H. Shaw, M. P. Thirlwell, and J. R. Ruedy, *ibid.*, **37,** 3981 (1977).

(5) M. Gibaldi, R. N. Boyes, and S. Feldman, *J. Pharm. Sci.*, **60,** 1338 (1971).

(6) C. E. Myers, R. Diasio, H. M. Eliot, and B. A. Chabner, *Cancer Treat. Rep.*, **3,** 175 (1976).

(7) W. Sadée and C. G. Wong, *Clin. Pharmacokinet.*, **2,** 437 (1977).

(8) I. D. P. Wootton, "Microanalysis in Medicinal Chemistry," 5th ed., Churchill and Livingstone, London, England, 1974, p. 104.

(9) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1975, p. 64.

(10) M. Gibaldi and D. Perrier, "Pharmacokinetics," 1st ed., Dekker, New York, N.Y., 1975, p. 145.

(11) K. B. Bischoff, R. L. Dedrick, and D. S. Zaharko, *J. Pharm. Sci.*, **59,** 149 (1970).

(12) B. Clarkson, A. O'Connor, L. Winston, and D. Hutchison, *Clin. Pharmacol. Ther.*, **5,** 581 (1964).

(13) C. Finn and W. Sadée, *Cancer Chemother. Rep.*, **59,** 279 (1975).

(14) H. O. Douglass and A. Mittelman, *Cancer*, **34,** 1878 (1974).

(15) L. E. Broder and P. P. Carbone, *Med. Pediatr. Oncol.*, **2,** 11 (1976).

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NOTES

Structural Elucidation of Adducts Formed by Ninhydrin with Indoles and Thiourea by ^{13}C-NMR Spectroscopy

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Abstract □ Condensation products were prepared by the reaction of ninhydrin with indole and 2,5-dimethylindole. The structures of these 1:1 adducts were assigned as 3-(2-hydroxy-2-indane-1,3-dionyl)indole and 3-(2-hydroxy-2-indane-1,3-dionyl)-2,5-dimethylindole, respectively, on the basis of spectral data including ^{13}C-NMR evidence. ^{13}C-NMR also was used to confirm the structure of a thiourea–ninhydrin adduct as a substituted thioindeno[1,2-*d*]imidazole-2,8-dione.

Keyphrases □ Ninhydrin—condensation products with indoles and thiourea, structural elucidation by NMR spectroscopy □ Heterocyclic adducts—cyclic ureides, condensation products of ninhydrin with indoles and thiourea, structural elucidation by NMR spectroscopy □ NMR spectroscopy—structural elucidation of condensation products of ninhydrin with indoles and thiourea

Interest in the heterocyclic adducts formed between ninhydrin (triketohydrindene hydrate, I) and aromatic amines (1, 2) and enamines (3) led to the investigation of the reaction products of this reagent with certain aromatic compounds whose structure might contain an enamine moiety. The structures of indole and 1-naphthylamine could be visualized as such; the N-1, C-2, and C-3 positions of indole and the NH_2, C-1, and C-2 positions of 1-naphthylamine possess the enamine triad (4). It was found that 1-naphthylamine forms a stable pentacyclic adduct with I, whose mode of cyclization has not been definitely established (5).

BACKGROUND

The ready formation of a 1:1 adduct between I and indole was first reported by Tomita and Fukagawa (6). However, the structure assigned by these investigators involved attack of the C-2 position of indole at the masked, central carbonyl group of ninhydrin to give II. This is in contrast to the expected reactivity of the C-3 position in indole (7, 8).

The reaction between I and indole was repeated by Roth and Kok (9); a stable, yellow adduct (1:1) was obtained with the same melting point as reported previously (6). These investigators (9) reported this adduct

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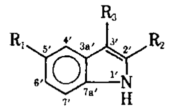


Table I—Carbon 13 Chemical Shifts of Substituted Indoles

Compound	R_1	R_2	R_3	C-3a′	C-7a′	C-2′	C-3′	C-5′	C-4′	C-6′
Indole[a]	H	H	H	128.8	136.1	125.2	102.6	122.3	121.3	120.3
2-Methylindole[a]	H	CH_3	H	129.9	137.1	135.7	100.4	121.1	120.0	119.9
3-Methylindole[a]	H	H	CH_3	129.2	137.3	122.7	111.4	122.3	119.4	119.6
2,5-Dimethylindole	CH_3	CH_3	H	128.7	135.1	134.2	98.4	126.5	121.1	118.4
III	H	H	—[b]	124.9	136.4	124.8	110.2		119.4[c]–121.9	
VII	CH_3	CH_3	—[b]	126.7	132.9	134.6	110.0	126.3	119.3	– 121.6[c]

[a] Taken from Ref. 12. [b] R_3 = 2-hydroxy-1,3-indandion-2-yl. [c] May be interchanged.

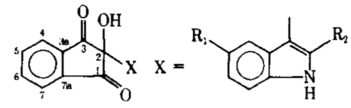


Table II—Carbon 13 Chemical Shifts of 2-Substituted Indandionyl Moieties

Compound	R_1	R_2	C-1 and C-3	C-2	C-4 and C-7	C-5 and C-6	C-3a and C-7a
I	X = OH		197.7	88.3	124.5	137.8	139.2
III	H	H	198.9	77.8	123.3	136.4	138.9
VII	CH_3	CH_3	198.7	78.0	123.0	136.0	139.7
XI	X = $NHCON(CH_3)_2$[a]		197.3	79.8	123.2	135.9	138.5

[a] Taken from Ref. 13.

as having Structure III, based on the interpretation of the PMR spectrum. However, a warning concerning the assignment of C-2 or C-3 substitutions in indole indicates that the effects of concentration and solvent polarity should be considered (10) when structural interpretations based on PMR evidence are made. However, these considerations were not cited in the report of Roth and Kok (9).

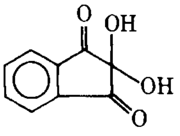
To establish unambiguously the structures of III and the products derived from 2,5-dimethylindole, thiourea, and I, ^{13}C-NMR spectroscopy was employed in the present study. Interest in the latter adduct (11) stems from the cyclic ureide (V) formed between urea and I (2). The nucleophilicity of the sulfur in thiourea prompted determination of the mode of cyclization; this investigation sought to distinguish between IV and VI or its [1,2-*d*]-isomer.

EXPERIMENTAL[1]

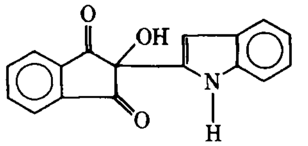
Melting points are uncorrected. All carbon spectra were decoupled with a 1-kHz wide noise bandwidth. All spectra were obtained in deuterated dimethyl sulfoxide; chemical shifts are reported relative to tetramethylsilane as the internal standard. Mass spectra were obtained on a double-focusing instrument[2] equipped with an electron-impact (70 ev) source and a direct-sample introduction probe.

Synthesis of 3-(2-Hydroxy-2-indane-1,3-dionyl)-2,5-dimethylindole (VII)—2,5-Dimethylindole (14.5 g, 0.01 mole) was dissolved in hot methanol (50 ml). To this solution was added I (1.78 g, 0.01 mole) in methanol (30 ml). The reaction mixture was warmed for 1 hr (80°) with stirring and set aside overnight. A volume of water equal to approximately one-third of the solution volume was added, and the solution was warmed and set aside for 2.5 hr. The crystals were filtered, washed with hot water, and dried.

The product weighed 2.67 g (87.5%), mp 200–205° dec. Recrystallization from methanol-water gave golden-yellow crystals, mp 198–202° dec.; IR: 3375 (OH), 3275 (NH), 1700, and 1748 (C=O) cm^{-1}; PMR: δ 10.97 (s, 1H, NH), 8.05 (s, 4H, aromatic), 7.29–6.75 (m, 3H, indole), 6.54 (s, 1H, OH), 2.31 (s, 3H, CH_3), and 2.26 (s, 3H, CH_3) (upon addition of deuterated water, the signals at δ 10.97 and 6.54 were diminished); mass spectrum: *m/e* 305 (M^+, 80%).



I



II

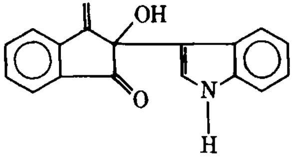
Anal.—Calc. for $C_{19}H_{15}NO_3$: C, 74.72; H, 4.95; N, 4.58. Found: C, 74.63; H, 4.92; N, 4.60.

Synthesis of 1,3,3a,8a-Tetrahydro-3a,8a-hydroxy-2-thioindeno[1,2-*d*]imidazole-2,8-dione (IV)—To 10 g of I (0.056 mole) in ~400 ml of hot water was added thiourea (4.25 g, 0.056 mole) dissolved in a minimum volume of water. The reaction mixture was stirred for 1 hr at 80°, cooled, and filtered. White crystals (12.0 g, 91%) were obtained, mp 236–240°. Recrystallization from water gave crystals with a melting point of 240–242°; IR: 3350–3100 (OH and NH) and 1715 (C=O) cm^{-1}; mass spectrum: *m/e* 236 (M^+, 100%).

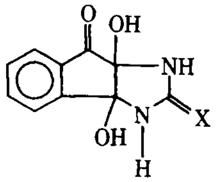
Anal.—Calc. for $C_{10}H_8N_2O_3S$: C, 50.85; H, 3.38; N, 11.86; S, 13.55. Found: C, 50.94; H, 3.44; N, 11.82; S, 13.66.

RESULTS AND DISCUSSION

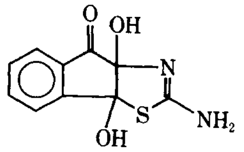
The carbon 13 chemical shifts of the carbon atoms of the adducts and some appropriate model compounds are shown in Tables I and II. Of critical significance are the peaks at 124.8 and 110.2 ppm, assigned to C-2′ and C-3′[3], respectively, of adduct III. This assignment is based on analogy



III



IV: X = S
V: X = O



VI

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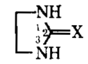
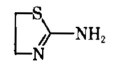
[1] IR spectra were taken on a Perkin-Elmer model 237 spectrophotometer and determined in potassium bromide pellets. PMR spectra were obtained on a Varian XL-100 NMR spectrometer with a Nicolet TT-100 Fourier transform accessory. The observing frequency for protons was 100 MHz; for carbon it was 25.2 MHz.

[2] Dupont 21-492.

[3] These positions refer to the indole moiety.

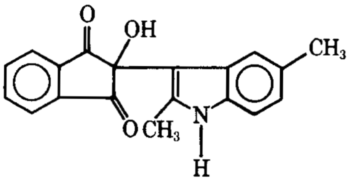
Table III—Carbon 13 Chemical Shifts of C═O and C═S

Compound	Chemical Shift of C-2
	
X: X = O	165.9
VIII: X = S	183.4
	
IX	159.0
V	158.3
IV	178.4

to the carbon 13 shifts assigned to simple C-2 and C-3 methylindoles shown in Table I. Such substituted carbons are shifted downfield by ~10 ppm. The data clearly indicate that this adduct is the 3-substituted indole (III). Similarly, the 2,5-dimethylindole–ninhydrin adduct was assigned Structure VII (Table I). In this case, the C-3′ position of the indole moiety absorbs at 110.0 ppm. The pertinent chemical shifts such as those of C-2′ are in agreement with those of the model compounds. This result also is consistent with the expected chemistry for a 2-substituted indole. In addition, the chemical shifts of the carbons in the indanedione moiety as shown in Table II support the structural assignments of adducts III and VII.

The reaction of I with thiourea in neutral aqueous solution gave a product that was assigned Structure IV. The C═S group previously has been difficult to identify positively by IR spectra (14). Moreover, the superior nucleophilicity of the thiocarbonyl sulfur over that of the nitrogen atom in this ambident nucleophile is well known (15, 16). Thus, the thiazoline structure (VI) could not be excluded since the PMR spectrum of the adduct in dimethyl sulfoxide-d_6 showed two exchangeable one-proton singlets at 9.70 and 9.48, a four-proton aromatic multiplet centered at 7.85, and a two-proton broad exchangeable singlet at 6.88, attributable to a primary amino group, as in VI, or two separate NH absorbances, as in IV (which have the same chemical shift).

These considerations necessitated examination of the ^{13}C-NMR data of this adduct along with closely related model compounds (Table III). Since the thiocarbonyl group in ethylenethiourea (VIII) absorbs at 183.4 ppm, and the corresponding carbon of 2-amino-2-thioazoline (IX) absorbs at 159.0, and the adduct in question absorbs at 178.4 (C═S), one concludes that this adduct has a thioureide structure (IV). In addition, a change from ureidocarbonyl to thiocarbonyl produces a downfield shift of ~18–20 ppm. This result is in accord with the differences in the chemical shift of C-2 (thiocarbonyl *versus* carbonyl) in uracil (17), V *versus* IV, and ethyleneurea (X) *versus* ethylenethiourea (VIII) in Table III.



VII

REFERENCES

(1) R. Shapiro and S. C. Agarwal, *J. Am. Chem. Soc.*, **90**, 474 (1968).

(2) R. Shapiro and N. Chatterjie, *J. Org. Chem.*, **35**, 447 (1970).

(3) N. Chatterjie, R. Shapiro, S. G. Quo, and R. Stephani, *Tetrahedron Lett.*, **1975**, 2535.

(4) G. R. Allen, Jr., "Organic Reactions," vol. 20, Wiley, New York, N.Y., 1973, p. 337.

(5) N. Chatterjie, Ph.D. dissertation, New York University, New York, N.Y., 1970.

(6) M. Tomita and T. Fukagawa, *Z. Physiol. Chem.*, **158**, 62 (1926).

(7) R. J. Sundberg, "The Chemistry of Indoles," Academic, New York, N.Y., 1970, pp. 1, 2, 39.

(8) J. E. Bloor and D. L. Bruen, *J. Am. Chem. Soc.*, **89**, 6835 (1967).

(9) H. J. Roth and W. Kok, *Arch. Pharm.*, **309**, 81 (1976).

(10) M. G. Reinecke, H. W. Johnson, Jr., and J. F. Sebastian, *Chem. Ind.*, **1964**, 151.

(11) N. Chatterjie and G. J. Alexander, *IRCS Med. Sci.*, **7**, 266 (1979).

(12) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic, New York, N.Y., 1972, pp. 266, 267.

(13) P. A. Crooks and T. Deeks, *Chem. Ind.*, **1975**, 793.

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Chapman and Hall, London, England, 1975, p. 399.

(15) J. P. Edwards, in "Organic Sulfur Compounds," vol. 2, N. Karasch, Ed., Pergamon, London, England, 1966, p. 291.

(16) A. H. Cook, J. D. Downer, and I. Heilbron, *J. Chem. Soc.*, **1948**, 1262, 2028.

(17) I. W. J. Still, N. Plavac, D. M. McKinnon, and M. S. Chauhan, *Can. J. Chem.*, **56**, 725 (1978).

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