Photochemical Cyclization of *N*-Butyl-*N'*-(9-acridinyl)thiourea

ANDRÉ De LEENHEER*, J. E. SINSHEIMER▲, and J. H. BURCKHALTER

Abstract □ The structure elucidation of 2-butylamino-1,3-thiazino-[4,5,6-*kl*]acridine, isolated after the reaction of 9-isothiocyanatoacridine with butylamine, is described. The same compound was found to be formed by photochemical cyclization of *N*-butyl-*N'*-(9-acridinyl)thiourea. The intense fluorescence of this cyclized product serves as the basis for the previously reported analysis of primary amines by reaction with 9-isothiocyanatoacridine.

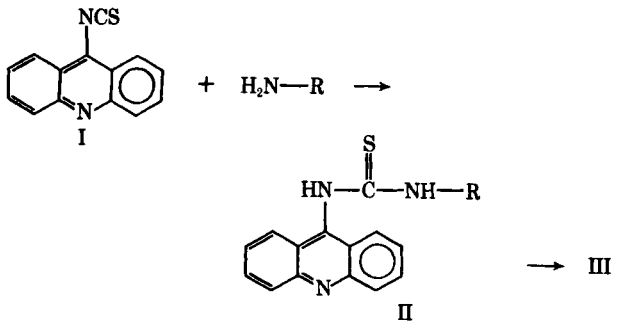
Keyphrases □ *N*-Butyl-*N'*-(9-acridinyl)thiourea—photochemical cyclization, isolation, characterization, structure identification of product □ 2-Butylamino-1,3-thiazino[4,5,6-*kl*]acridine—isolation, characterization, structure identification as photochemical cyclization product of *N*-butyl-*N'*-(9-acridinyl)thiourea □ Amines, primary—fluorescence measurement of cyclized 2-butylamino-1,3-thiazino[4,5,6-*kl*]acridine

Fluorescent analysis of primary aliphatic amines by reaction with 9-isothiocyanatoacridine (I) was previously reported from these laboratories (1). The thioureas (II) formed in this procedure are not stable and yield new intensely fluorescent compounds (III) whose fluorescence can be related to the concentration of the original amines (Scheme I).

The present study is concerned with the structure elucidation and synthesis of a typical compound (III) resulting after the initial reaction of 9-isothiocyanatoacridine with butylamine to form *N*-butyl-*N'*-(9-acridinyl)thiourea (II).

EXPERIMENTAL[1]

Preparation of 9-Isothiocyanatoacridine (I)—9-Chloroacridine (1.15 g., 0.005 mole) was dissolved in 200 ml. of dimethylformamide, and 1.482 g. (0.015 mole) of potassium thiocyanate was added while the mixture was stirred. The reaction was heated in an oil bath, which was slowly brought to 90° and kept at that temperature for 45 min. The mixture was cooled overnight in a refrigerator, the precipitated KCl was removed by filtration, and dimethylformamide was removed in a rotary evaporator *in vacuo*. Crystallization from acetone gave an 88% yield of yellow needles, m.p. 129° [lit. (2) m.p. 131–132°]; $\nu_{max.}^{KBr}$ 2050–2150 (N=C=S), 1540, 1505, 1410 (aromatic), 1180 (C=S), and 455 cm.$^{-1}$ (1,2-substituted phenyl); δ ($CDCl_3$) 7.0–8.0 (m, aromatic H).



Scheme I

Preparation of *N*-Butyl-*N'*-(9-acridinyl)thiourea (II)—9-Isothiocyanatoacridine (0.708 g., 0.003 mole) was dissolved in 45 ml. of toluene, and 0.277 g. (0.004 mole) of *n*-butylamine was added. The stirred mixture was heated at reflux for 15 min. in an oil bath at 110–120°. The solution was cooled, and the yellow precipitate was collected on a filter and washed with small amounts of toluene. Recrystallization from 95% ethanol afforded an 84% yield of yellow plates, m.p. 180–181°; $\nu_{max.}^{KBr}$ 3480, 3140 (NH), 3050 (aromatic CH), 2910 (aliphatic CH), 1610, 1580 (aromatic), 1530 (CNH), 1470 (aliphatic CH_2), 1260 (aromatic CH), 1210 (C=S), 1150, 1080 (C—N), 450 (1,2-substituted phenyl), and 425 cm.$^{-1}$ (aliphatic CH_2); δ (CF_3COOH) 1.00 (3H, t, CH_3), 1.62 (4H, m, CH_2—CH_2), 3.77 (2H, t, N—CH_2), and 7.7–8.4 (10H, m, aromatic H and NH); mass spectrum (50 ev., 200°) 309 (M), 275 (M—H_2S), 252 (M—C_4H_9), 236 (M—C_4H_9—NH_2), 219 (M—H_2S and C_4H_8), 211 (M—C_4H_9N and HCN), 205 (M—H_2S and C_4H_8N), and 194 (M—C_4H_9—NCS base peak).

Isolation of 2-Butylamino-1,3-thiazino[4,5,6-*kl*]acridine (III)—A mixture of 40 mg. (0.0001 mole) of *N*-butyl-*N'*-(9-acridinyl)thiourea (II), 1 ml. of 0.2 *N* NaOEt in ethanol, and 15 ml. of absolute ethanol was heated at reflux for 2.5 hr. The reaction mixture was cooled, 1 ml. of 10% aqueous HCl was added, and the solvent was removed on a rotary evaporator *in vacuo*. The product was isolated by GLC, in which 40-μl. samples of an extract of 10 mg. of reaction product in 10 ml. of absolute ethanol were chromatographed as outlined here. The samples of product collected from multiple runs were combined, dissolved in 0.5 ml. of absolute ethanol, and rechromatographed to obtain material giving the following properties: m.p. 195–196°; $\lambda_{max.}^{95\%\ EtOH}$ (ε) 219 (102,000), 239 (89,900), 266 (150,000), 456 (25,000), 460 (30,100), and 485 nm. (19,200); $\lambda_{min.}$ 232, 242, 444, and 477 nm.; fluorescence $\lambda_{act.}^{95\%EtOH}$ 270 nm. and $\lambda_{fluor.}$ 510 nm.; $\nu_{max.}^{KBr}$ 3450, 3180 (NH), 3050 (aromatic CH), 2910 (aliphatic CH), 1605, 1580 (phenyl), 1530 (NH and CN), 1470 (aliphatic CH_2), 1260 ($C_{aromatic}$—N), 1155 (CN), 445 (1,2,3-substituted phenyl), and 462 cm.$^{-1}$ (1,2-substituted phenyl); δ (CF_3COOH) 8.5–7.0 (m, 8, aromatic H and NH), 3.77 (2H, s, N—CH_2), 1.66 (4H, m, CH_2CH_2), and 1.10 (3H, s, CH_2); mass spectrum (70 ev., 180°) *m/e* 307 (M) [M + 1 (19.4%) and M + 2 (5.9%)], 278 (M—C_2H_5), 274 (M—SH), 264 (M—C_3H_7), 251 (M—C_4H_8), 236 (M—C_4H_8 and NH), 224 (M—C_4H_8 and HCN, base peak), 191 (M—C_4H_8, HCN and SH), and 164 (M—C_4H_8, HCN, SH, and HCN); high-resolution mass spectrum *m/e* (calculated formula) 308.11634 (C_{17}-^{13}C-$H_{17}N_3S$, 308.11792), 307.11424 ($C_{18}H_{17}N_3S$, 307.11426), 274.13650 ($C_{18}H_{16}N_3$, 274.13442), 251.05069 ($C_{14}H_9N_3S$, 251.05168), 225.04194 (C_{12}-^{13}C-H_8N_2S, 225.04414), 224.03825 ($C_{13}H_8N_2S$, 224.04080), 223.03024 ($C_{13}H_7N_2S$, 223.03296), and 190.05944 ($C_{13}H_7N_2$, 191.06093).

Photochemical Preparation of 2-Butylamino-1,3-thiazino[4,5,6-*kl*]acridine (III)—A solution of 100 mg. (0.0003 mole) of *N*-butyl-*N'*-(9-acridinyl)thiourea (II) in 200 ml. of cooled 95% ethanol was transferred to a Hanovia glass immersion flask. A 450-w., high intensity, mercury vapor lamp[2] was immersed into the solution; irradiation was maintained for 1 hr., during which time the solution changed from yellow to red-brown with an intense green fluorescence near the surface. The ethanol was removed on a rotary evaporator *in vacuo*, and the residue was triturated with petroleum ether followed by recrystallization from ethyl acetate–cyclohexane (1:1) to yield a red compound in 50% yield, m.p. 195–196°. IR,

[1] Melting points, observed by means of a Mel-Temp apparatus, are uncorrected. The instruments employed were: IR, Perkin-Elmer model 337; NMR, Varian A-60-A; UV, Beckman DK-2A; fluorometer, American Instrument Co. 4-8016; mass spectrometers, AEI MS902, Hitachi RMU-6-D, and CEC-21-110B; and gas chromatograph, Hewlett Packard 5750.

[2] Hanovia 679A36 with Vycor 7910 filter.

TLC, and GLC properties were the same as the isolated product III.

Chromatography of 2-Butylamino-1,3-thiazino[4,5,6-*kl*]acridine—TLC was accomplished on activated (105° for 1 hr.) cellulose (250 μ) using a solvent system of dimethylformamide–water–28% ammonia (5:13:3 by volume). An R_f value of 0.08 was obtained for a single development while R_f 0.28 was obtained upon redevelopment with detection by yellow-green fluorescence under UV light.

GLC was performed in two systems under the following conditions: oven temperature, 250°; injector temperature, 260°; flame-ionization detector temperature, 260°. In System I [5.2% OV-17 on Gas Chrom Q 80–100 in a 1.82-m. × 0.63-cm. (6-ft. × 0.25-in.) glass column with a helium flow of 10.5 cm. sec.$^{-1}$], R_t was 46.4 min. (N = 2460). In System II [3.8% UCC-W98 on Diatoport S 80–100 in a 1.23-m. × 0.63-cm. (4-ft. × 0.25-in.) glass column with a helium flow of 11.0 cm. sec.$^{-1}$], R_t was 15.5 min. (N = 2082).

RESULTS AND DISCUSSION

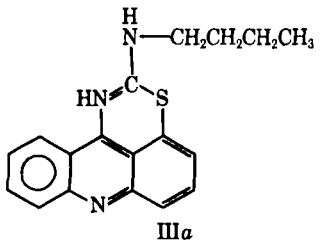
9-Isothiocyanatoacridine was prepared by reaction of 9-chloroacridine with potassium thiocyanate in dimethylformamide at 90°. In this manner, higher yields (88%) were obtained as compared to the authors' use of Kristian's (2) silver thiocyanate procedure (58% yield) or the reaction of 9-aminoacridine and thiophosgene (20% yield). However, control of temperature is important because IR and mass spectral analyses indicated the presence of considerable impurities in the reaction product isolated at higher temperatures. Optimal synthesis conditions were developed by following the reaction with GLC on 3.8% UCC-W98. Both the appearance of a 9-isothiocyanatoacridine peak [R_t 4 min. 29 sec. (220°); 1 min. 36 sec. (250°)] and the disappearance of a 9-chloroacridine peak [R_t 1 min. 46 sec. (220°); 46 sec. (250°)] were useful in monitoring the reaction.

The *N*-butyl-*N'*-(9-acridinyl)thiourea required for this study was prepared in toluene in contrast to the use of absolute alcohol as the solvent in the analytical procedure (1).

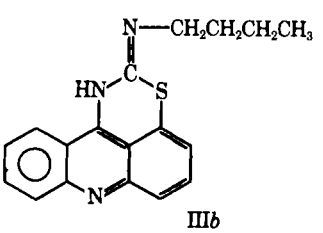
The final product, III, was originally formed in a manner analogous to the analytical procedure with isolation by preparative GLC. However, during such preparations, it was noted that the yields of III decreased when exposure to light was decreased. Subsequently, it was confirmed that the same compound (identical IR, TLC, and GLC properties) was formed photochemically when an alcoholic solution of the butyl acridinylthiourea was exposed to a high intensity mercury lamp.

The UV, visible, and fluorescence properties of Compound III indicated an extension of conjugation with respect to the original thiourea. A loss of two hydrogens was indicated by a mass spectral molecular ion of 307. High-resolution determination of this ion was consistent with a molecular formula of $C_{18}H_{17}N_3S$. The 12 sites of unsaturation demanded by this formula were assigned as eight double bonds in four rings, an assignment involving cyclization to form an additional ring rather than double-bond formation in the thiourea side chain. This choice was based upon 1,2,3-trisubstituted phenyl absorption in the IR and an NMR spectra showing eight protons in the 8.5–7.08 region (seven aromatic H and one NH) in contrast to 10 protons (eight aromatic and two NH) for *N*-butyl-*N'*-(9-acridinyl)thiourea. The lack of olefinic protons in the IR and NMR spectra also supported the cyclized structure.

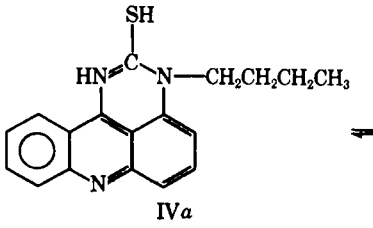
Cyclizations through both sulfur (III*a* ⇌ III*b*) and nitrogen (IV*a* ⇌ IV*b*) were considered.



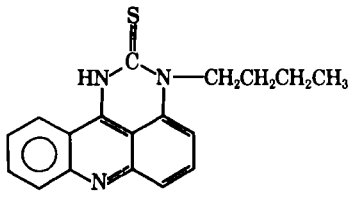
III*a*



III*b*

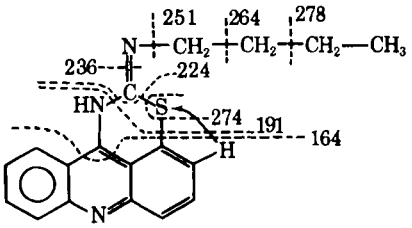


IV*a*



IV*b*

The mass spectral fragmentation pattern was in favor of III*a* ⇋ III*b* and could be envisioned as follows:



Metastable peaks at 205.2 and 199.9 confirmed one-step decompositions in the sequence 307 to 251 and then to the base peak at 224. It is the 224 fragment that particularly supports an endocyclic sulfur.

The peaks at 251, 224, and 191 were intense enough to confirm the formulas of these fragments by high-resolution mass spectrometry. Although there was some loss of SH to yield a 274 ion, it did not necessarily indicate an exocyclic sulfur because C—S—C structures have been shown to undergo such a loss (3).

The spectral properties of proposed Structure III*a* are of interest. The compound is efficient in its absorbance of energy, as indicated by high molecular absorptivity values in the UV range. It is also intensely fluorescent. The fluorescence intensity of the compound measured in 95% ethanol ($\lambda_{act.}$ 270 nm. and $\lambda_{fluor.}$ 510 nm.) was about 70% of that of 9-aminoacridine ($\lambda_{act.}$ 260 nm. and $\lambda_{fluor.}$ 510 nm.), which is one of the most fluorescent compounds reported (4). *N*-Butyl-*N'*-(9-acridinyl)thiourea (II) possesses about 3% of the fluorescence of 9-aminoacridine. This increase in fluorescence serves as the basis of our procedure for the analysis of primary amines (1).

REFERENCES

(1) J. E. Sinsheimer, D. D. Hong, J. T. Stewart, M. L. Fink, and J. H. Burckhalter, *J. Pharm. Sci.*, **60**, 141(1971).

(2) P. Kristian, *Chem. Zvesti*, **15**, 641(1961).

(3) P. Brown and C. Djerassi, *Angew. Chem.*, **6**, 493(1967).

(4) A. Albert, "The Acridines," 2nd ed., Edward Arnold, London, England, 1966, p. 289.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 14, 1971, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication September 27, 1971.

Supported in part by Research Grant AI 05817 from the National Institutes of Health.

The authors thank Mr. F. A. MacKellar, Department of Chemistry, University of Michigan, and Dr. William L. Budde, Purdue Mass Spectrometry Center (U.S. Public Health Service Grant FR-00354), for the mass spectral data and assistance in their interpretation.

* Present address: Laboratorium voor Toxicologie, University of Gent, Gent, Belgium.

▲ To whom inquiries should be directed.