

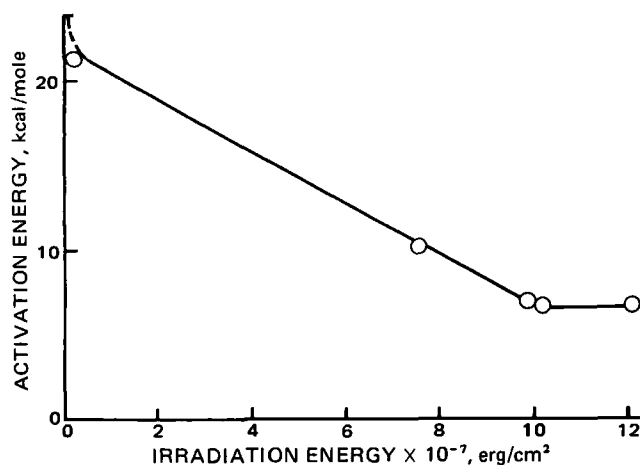


Figure 14—Effect of UV intensity on the activation energy.

These values were smaller by one or two orders of magnitude in comparison with the UV intensity ratio of 579, irradiated on each sample. In this respect, the elevation of temperature could be verified again to have a synergistic effect on the chemical degradation of ubidecarenone. The effect of UV irradiation energy on the activation energy calculated from the slope of each regression line is shown in Fig. 14. The activation energy linearly decreased up to $\sim 1.0 \times 10^8$ erg/cm²; with values larger than this it was no longer dependent on the UV intensity. It should be pointed out that even under very low intensity (in the case of filter VY-42), the activation energy approached a finite value of 21.3 kcal/mole, which is within the usual range of activation energies for hydrolysis or oxidation (21).

REFERENCES

- (1) S. R. Byrn, *J. Pharm. Sci.*, **65**, 1 (1976).
- (2) T. Eble and E. R. Garrett, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 536 (1954).
- (3) L. J. DeMerre and C. Wilson, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**,

129 (1956).

- (4) E. E. Kaminski, R. M. Cohn, J. L. McGuire, and J. T. Carstensen, *J. Pharm. Sci.*, **68**, 368 (1979).
- (5) Y. Matsuda and Y. Minamida, *Chem. Pharm. Bull.*, **24**, 2228 (1976).
- (6) Y. Matsuda, H. Inouye, and R. Nakanishi, *J. Pharm. Sci.*, **67**, 196 (1978).
- (7) Y. Matsuda and M. Mihara, *Chem. Pharm. Bull.*, **26**, 2649 (1978).
- (8) Y. Matsuda and M. Itoh, *Asian J. Pharm. Sci.*, **1**, 107 (1979).
- (9) Y. Matsuda and R. Masahara, *Yakugaku Zasshi*, **100**, 953 (1980).
- (10) "Shin-iyakuhin no Kikaku-Shikensho Kaisetsu," Society of Japanese Pharmacopeia ed., Yakugyo Zihosha Co., Tokyo, Japan, 1978, p. 123.
- (11) Y. Matsuda and Y. Minamida, *Yakugaku Zasshi*, **96**, 425 (1976).
- (12) R. S. Hunter, *J. Opt. Soc. Am.*, **38**, 661 (1948).
- (13) K. Shibata, "Spectrophotometry and Spectrophotometer" (in Japanese), Kohdansha Co., Tokyo, Japan, 1974, p. 159.
- (14) E. I. Stearns, *Am. Dyestuff Rep.*, **40**, 563 (1951).
- (15) Y. Matsuda, T. Itooka, and Y. Mitsuhashi, *Chem. Pharm. Bull.*, **28**, 2665 (1980).
- (16) H. E. Zimmerman and W. R. Elser, *J. Am. Chem. Soc.*, **91**, 887 (1969).
- (17) O. L. Chapman and R. D. Lusa, *J. Am. Chem. Soc.*, **92**, 6352 (1970).
- (18) J. Saltiel, J. T. D'Agostino, O. L. Chapman, and R. D. Lusa, *J. Am. Chem. Soc.*, **93**, 2804 (1971).
- (19) P. de Mayo, *Acc. Chem. Res.*, **4**, 41 (1971).
- (20) R. E. Notari and S. M. Caiola, *J. Pharm. Sci.*, **58**, 1203 (1969).
- (21) L. Kennon, *J. Pharm. Sci.*, **53**, 815 (1964).

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Determination of Busulfan in Plasma by GC-MS with Selected-Ion Monitoring

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Abstract □ A GC-MS technique with selected-ion monitoring is described for the determination of busulfan in plasma. Busulfan is extracted from plasma with methylene chloride and converted to 1,4-diiodobutane. Analysis by GC-MS with selected-ion monitoring (m/z 183) gave a relative standard deviation of $\pm 4.3\%$ ($n = 5$) at the 10-ng/ml level.

Keyphrases □ Busulfan—determination in human plasma, GC-MS with selected-ion monitoring of 1,4-diiodobutane □ GC-MS analysis—selected-ion monitoring, determination of busulfan in human plasma

The alkylating agent, busulfan, 1,4-butanediol dimethanesulfonate, is the drug of choice in the treatment of chronic myelogenous leukemia. The drug has been in clinical use since the 1950's, but the fate of the drug in humans has only been studied by administering radioactively labeled compound and measuring total radioactivity in plasma and urine (1, 2). This paper describes the conversion of busulfan to 1,4-diiodobutane, and the subse-

quent quantitation of this material by GC-MS with selected-ion monitoring.

EXPERIMENTAL

Synthesis of 1,5-Pentanediol Dimethanesulfonate (Internal Standard)—Methanesulfonic anhydride (9 g) was added carefully to a stirred mixture of 1,5-pentanediol (5.5 g) in pyridine-methylene chloride (40 ml, 1:1). After stirring overnight at 25° the mixture was filtered, and the organic phase was washed with water. The organic phase was evaporated to ~ 5 ml and left at 4° for 48 hr. The crystals which formed were separated, washed with ice-cold water, and dried (yield: 9%); mp 35° [lit. (3) 34–35°]. The compound was identified by GC-MS¹ after conversion to the corresponding 1,5-diiodo derivative according to the procedure given below. There were prominent peaks at m/z 324 (M^+ , 8%), 199 (5), 197 (53), 169 (5), 155 (26), 70 (8), and 69 (100).

Conversion of Busulfan to 1,4-Diiodobutane—Busulfan was con-

¹ LKB 2091; the ionizing energy was 70 eV.

Table I—Stability of Busulfan at 37.0°^a

Medium	<i>t</i> _{1/2} (±SE), hr
Whole blood	8.7 ± 0.4 (<i>n</i> = 10)
Plasma	12.2 ± 0.1 (<i>n</i> = 10)
Phosphate buffer (pH 7.0)	16.0 ± 0.7 (<i>n</i> = 7)

^a Busulfan concentration: 2.8×10^{-4} M.

verted to 1,4-diiodobutane using 1 M sodium iodide in acetone (0.1 ml), with a reaction time of 20 min at 70°. After addition of *n*-hexane (0.05 ml) and water (0.1 ml), an aliquot (1–2 μl of hexane) was removed for analysis.

Determination of Partition Coefficient—The distribution of busulfan was studied using phosphate buffer (pH 7.0, $\mu = 0.1$) as the aqueous phase and methylene chloride as the organic phase with an equilibrium time of 30 min at $25.0 \pm 0.1^\circ$. The concentration of busulfan in the organic phase was determined after addition of the internal standard, evaporation, and conversion to 1,4-diiodobutane as above. The concentration of busulfan in the aqueous phase was determined after transfer into methylene chloride by extraction of the aqueous phase three times with equal volumes of the solvent. The analyses were performed by GC–FID².

Determination of Rate Constants for the Formation of 4-iodo-1-butanol Methanesulfonate (I) and 1,4-Diiodobutane—At appropriate times 1.00-ml samples were withdrawn from an acetone solution of busulfan (4×10^{-3} M) in 1 M sodium iodide at $50.0 \pm 0.5^\circ$. The reaction was stopped by rapid cooling (-70°), and the acetone was evaporated to near dryness. The residue was mixed with 1.00 ml of methylene chloride and 1.00 ml of water. The concentration of 1,4-diiodobutane was determined by mixing 0.100 ml of the organic phase with 1,5-diiodopentane and analyzing by GC–FID. The concentrations of busulfan and I were determined using the following procedure. The methylene chloride phase (0.500 ml) was evaporated to dryness, the residue was mixed with 0.500 ml of ethanol–water (1:1), and an aliquot (0.100 ml) was injected into the liquid chromatograph³. The fractions containing busulfan and I were collected, mixed with the internal standard and extracted with methylene chloride. The organic phase was evaporated to dryness, and busulfan and I were converted to 1,4-diiodobutane and analyzed by GC.

The mass spectrum of I was consistent with the expected structure. Peaks were located at *m/z* 278 (*M*⁺, 1%), 183 (1), 151 (10), 86 (13), 84 (20), 79 (14), 56 (5), 55 (100), and 51 (9). The mass spectrum of the prepared 1,4-diiodobutane was identical with that of a commercially available reference compound. Peaks were located at *m/z* 310 (*M*⁺, 2%), 184 (5), 183 (81), 155 (10), 137 (8), 135 (8), 56 (10), and 55 (100).

Determination of Busulfan in Plasma—Plasma (1.00 ml) was mixed with 0.100 ml of the internal standard (1.00 μg/ml) in acetone and extracted with methylene chloride (4.00 ml) for 30 min using a mechanical shaker (100 strokes/min). The organic phase was separated and evaporated to dryness, and the derivatization was performed using the aforementioned procedure. The analysis was carried out by GLC–MS⁴ focusing at *m/z* 183 (1,4-diiodobutane) and *m/z* 197 (1,5-diiodopentane).

RESULTS AND DISCUSSION

Stability of Busulfan—The stability of busulfan in whole blood and plasma is lower than in phosphate buffer, pH 7.0 (Table I). Busulfan differs in this respect from the nitrogen mustards, chlorambucil and melphalan, where an increased stability has been observed in samples containing albumin (4, 5). The stability of busulfan was also studied in plasma at a concentration of 4×10^{-7} M and was not significantly different from that observed at 2.8×10^{-4} M. The degradation of busulfan in human blood samples was minimized by rapidly cooling the blood to 4° and freezing the plasma fraction (-20°) within 1 hr after the blood collection.

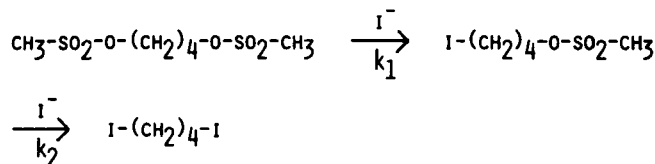
Extraction—Methylene chloride was used in the extraction of busulfan because of its favorable extraction properties (*k*_d = 28.7) and high

Table II—GC of busulfan^a

Busulfan Injected, μg	Peak Height Ratio ^b	Column Temperature
1	0.80 ± 0.11	190°
0.2	0.23 ± 0.04	190°
1	0.97 ± 0.09	175°
0.2	0.44 ± 0.13	175°

^a The column was 3% OV-17 on 100–120 Gas Chrom Q (1.5 m) with injector and detector (FID) temperatures of 210° and 270°, respectively. The carrier gas flow was adjusted to give the same retention time (5.2 min) for busulfan at 190° and 175°.

^b The mean peak height ratio of busulfan to 9-bromophenanthrene (±SD; *n* = 6). The busulfan–9-bromophenanthrene ratio was kept constant.



Scheme I—Reaction of busulfan with sodium iodide.

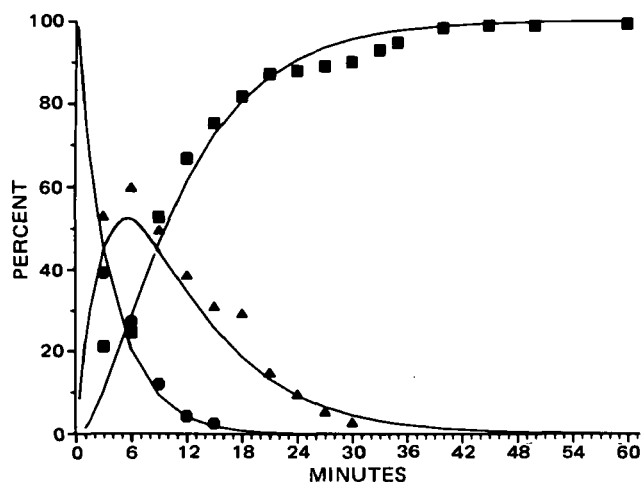


Figure 1—Time course for the reaction of busulfan with 1 M sodium iodide in acetone at 50°. The solid lines are constructed from the rate constants obtained by nonlinear regression analysis. Key: (●) busulfan; (▲) 4-iodo-1-butanol methanesulfonate; (■) 1,4-diiodobutane.

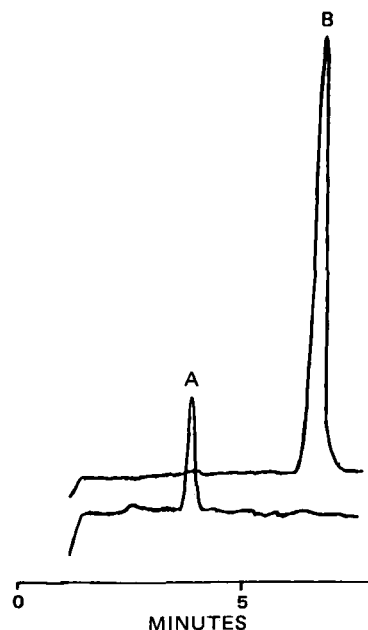


Figure 2—Chromatogram obtained from plasma containing 10 ng of busulfan/ml using selected-ion monitoring with a 1.5-m column packed with 10% SP-2401 at 140°. Key: (A) busulfan as 1,4-diiodobutane; (B) 1,5-pentanediol dimethanesulfonate as 1,5-diiodopentane.

² Varian 3700; the column (1.5-m × 2-mm i.d.) was packed with 10% SP-2401 on 100–120 Supelcoport and was operated at 130°.

³ The column (150-mm × 4-mm i.d.) was packed with LiChrosorb RP-8. Ethanol–water (1:1) was used as the mobile phase. The detection was performed by an Altex model 153 detector measured at 254 nm (1,4-diiodobutane and I) and by an LDC Refracto Monitor III (busulfan). The capacity factors were 0.3 (busulfan), 0.9 (I), and 4.6 (1,4-diiodobutane).

⁴ LKB 2091 with an ionizing energy of 70 eV. The column (1.5-m × 2-mm i.d.) was packed with 10% SP-2401 on 100–120 Supelcoport and was operated at 140° using a helium flow rate of 20 ml/min. The injector and the ion source temperatures were 230° and 270°, respectively.

volatility, being readily evaporated prior to the derivatization step. Using a $V_{\text{org}}/V_{\text{aq}}$ ratio of $4.98 \pm 1\%$ of busulfan ($n = 5$) was extracted from plasma (busulfan concentration 50 ng/ml).

GC of Intact Busulfan—The peak height ratios of busulfan to 9-bromophenanthrene obtained after injections of different amounts of the compounds at two column temperatures were drastically reduced when lower amounts of busulfan were chromatographed; in all cases poor precision was obtained (Table II). Analysis of busulfan at the lower temperature (175°), keeping the retention time the same by adjustment of the carrier gas flow, gave the highest peak height ratio, which indicates that the results obtained are primarily due to degradation of busulfan in the chromatographic system and not to adsorption phenomena (6). This assumption is also supported by the fact that when using different batches of OV-17 column packing material, multiple asymmetric peaks were occasionally observed.

Conversion of Busulfan to 1,4-Diiodobutane—The reaction of busulfan with sodium iodide proceeds according to Scheme I. The reaction was performed in acetone since nucleophilic substitution reactions are known to be rapid in this solvent (7). The time course for busulfan, I, and 1,4-diiodobutane using 1 M sodium iodide in acetone is given in Fig. 1. Evaluation of the apparent first-order rate constants by nonlinear regression analysis gave $k_1 = 0.266 \pm 0.013$ and $k_2 = 0.124 \pm 0.008 \text{ min}^{-1}$. Since the ratio between the constants is ~ 2 , it follows that the methanesulfonate ester group of busulfan and that of I have similar reactivity (3). A quantitative reaction was obtained after 20 min using 1 M sodium iodide and a temperature of 70° .

Chromatographic Properties—1,4-Diiodobutane had excellent GC properties giving a symmetric peak (Fig. 2). No indications of decomposition in the chromatographic system were observed.

Detection, Selectivity, and Precision—The minimum detectable concentration (MDC) value obtained by electron-capture detection (ECD) was ~ 10 times lower than that obtained by selected-ion monitoring (SIM), 0.6×10^{-16} and 5.7×10^{-16} mole/sec, respectively⁵. How-

ever, analysis of plasma samples revealed that the higher sensitivity of the ECD could not be utilized because of interfering peaks in the chromatograms. The blanks varied considerably between patients and, in most cases, it was not possible to perform quantitations < 10 ng/ml. Since the plasma peaks after administration of therapeutic doses (2 mg) of busulfan are 20–30 ng/ml, meaningful pharmacokinetic studies require determinations in the low nanogram range. A chromatogram obtained from plasma using SIM is given in Fig. 2. The standard curve obtained from plasma using SIM was linear within the range studied (10–400 ng/ml). A least-squares analysis gave a correlation coefficient of 0.9997, a slope of $2.12 \times 10^{-2} \pm 0.03 \times 10^{-2}$, and an intercept of $4.7 \times 10^{-2} \pm 7.6 \times 10^{-2}$. The relative standard deviation was $\pm 2.6\%$ at 100 ng/ml and $\pm 4.3\%$ at 10 ng/ml ($n = 5$).

REFERENCES

- (1) M. V. Nadkarni, E. G. Trams, and P. K. Smith, *Cancer Res.*, **19**, 713 (1959).
- (2) H. Vodopick, H. E. Hamilton, and H. L. Jackson, *J. Lab. Clin. Med.*, **73**, 266 (1969).
- (3) R. F. Hudson, G. M. Timmis, and R. D. Marshall, *Biochem. Pharmacol.*, **1**, 48 (1958).
- (4) H. Ehrsson, U. Lönnroth, I. Wallin, M. Ehrnebo, and S. O. Nilsson, *J. Pharm. Pharmacol.*, **33**, 313 (1981).
- (5) H. Ehrsson and U. Lönnroth, *J. Pharm. Sci.*, **71**, 826 (1982).
- (6) K. Grob, *J. High Resol. Chromatogr.*, **3**, 585 (1980).
- (7) A. J. Parker, *Chem. Rev.*, **69**, 1 (1969).

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Steroidal Thiourea and Thiazoline Derivatives: Synthesis and *In Vitro* Effects on Bovine Pancreatic Ribonuclease Activity

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Received February 24, 1982, from the *Pharmaceutical Chemistry Department, Faculty of Pharmacy, the †Department of Applied Medical Chemistry, Medical Research Institute, University of Alexandria, Egypt and the §Laboratoire de Chimie des Hétérocycles d'Intérêt Biologique, Université d'Aix-Marseille II, Faculté de Médecine Nord, 13326 Marseille, Cédex 15, France. Accepted for publication August 16, 1982.

Abstract □ Two novel series of steroidal derivatives containing various thiourea and substituted thiazoline moieties attached to the 2- or 4-position of estrone were synthesized and examined for *in vitro* effect on bovine pancreatic ribonuclease activity. All compounds studied exhibited a catabolic activity. The steroidal thiazoline derivatives were more potent activators of ribonuclease than the steroidal thioureas.

Keyphrases □ Steroids—thiourea and thiazoline derivatives, synthesis, *in vitro* effect on bovine pancreatic ribonuclease activity □ Synthesis—steroidal thiourea and thiazoline derivatives, *in vitro* effect on bovine pancreatic ribonuclease activity □ Catabolic activity—steroidal thiourea and thiazoline derivatives, *in vitro*, bovine pancreatic ribonuclease activity

Recent reports from this laboratory have described the synthesis and pharmacological properties of a variety of androgenic and estrogenic thiosemicarbazones (1),

acylhydrazones (2–5), and several steroidal heterocycles (6, 7). Further interest in structure–activity relationships (SAR) of steroidal heterocyclics prompted the preparation of XVIII–XXXII (Scheme I) to evaluate the changes in the endocrinological activity caused when the 2- or 4-position of estrone-3-methyl ether is blocked by variously substituted thiazoline moieties. Some of the steroidal thioureas (VIII–XVI), prepared as starting materials, and the thiazoline derivatives were found to possess catabolic-like properties as indicated from their *in vitro* effect on the activity of bovine pancreatic ribonuclease.

RESULTS AND DISCUSSION

Synthesis—The designed compounds (XVIII–XXXII) were prepared in accordance with the sequence of reactions shown in Scheme I. The 2-