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Keyphrases

Benzoic acid solubilization
Solubilization with *n*-alkylpolyoxyethylene surfactants—temperature effect
Equilibrium constants controlling solubilization
Micellar solubilization
Distribution coefficient
Dialysis studies
UV spectrophotometry—analysis

Synthesis and Properties of the Antileukemic Agent 5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide

By Y. FULMER SHEALY, CHARLES A. KRAUTH*, LEE B. HOLUM, and WILLIAM E. FITZGIBBON

The antileukemic agent 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (II, NSC-82196) is an unstable compound that is easily convertible to an isomeric transformation product. By taking appropriate precautions, however, the triazene may be prepared in good yield, with very little of the transformation product as contaminant, and may be stored for long periods at low temperatures. The quality of specimens of II may be estimated from its infrared spectrum and that of its transformation product.

In the mouse lymphoid leukemia L1210 test system (1), 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (II) has demonstrated excellent activity. Some of the animals treated with large single doses of this compound survived until the experiments were terminated 4–8 months after treatment. From the cell-kill kinetic studies of Skipper, Schabel, and Wilcox (2, 3), it may be concluded that complete eradication of leukemic cells must have occurred in these survivors. Compound II is unstable and is readily converted to an isomeric product that is not active against leukemia L1210. In a preliminary communication (1) the authors reported, without experimental details, the preparation of II from 5-diazoimidazole-4-carboxamide (I) and bis(2-chloroethyl)amine (III). In this report complete details are recorded on the preparation of II, including improvements; and some of its properties and the precautions that must be observed during the preparation, storage, and use of this compound are described more fully.

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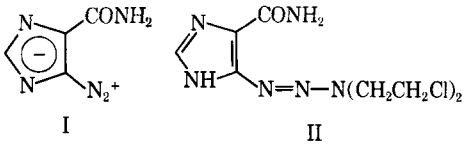

I II

$(ClCH_2CH_2)_2NH$ $[C_8H_{12}N_6OCl^+]Cl^-$

III IV

Bis(2-chloroethyl)amine free base (III) was extracted from an alkaline solution into either ethyl acetate or dichloromethane. In the procedure mentioned in the preliminary report (1) an ethyl acetate solution of III was diluted with methanol, the reaction of I and III was carried out in a 2 : 1 mixture of ethyl acetate and methanol, and the product was precipitated by the addition of a very large volume of petroleum ether (b.p. 30–60°). The preparative procedure has subsequently been improved by employing only methanol as the reaction medium (after removal of the extraction solvent from III) and by establishing an optimum temperature of about 15–20°. Compound II crystallizes directly from the reaction mixture and is obtained in higher yield (65–75%) and in improved quality without the need for large volumes of petroleum ether. Since dichloromethane is more easily volatilized, it is the preferred solvent for extracting III.

Although compound II is only slightly soluble in water and is sparingly soluble in methanol, prolonged stirring of a suspension of II in either solvent gives a solution from which a new compound is isolated. This transformation product (IV) has the same composition as II, but half of the chlorine is ionic as was shown by titration with silver nitrate. A suspension of the triazene (II) in a solution of the Bratton-Marshall reagent (4) gives a positive test upon acidification; under comparable conditions IV does not give a positive Bratton-Marshall test. Possible structures for the transformation product (IV) include aziridinium, *v*-triazolinium, and piperazinium salts formed by internal alkylation. The change of II to IV also occurs in dimethylsulfoxide solution and even in the solid state at room temperature. Infrared spectra showed, as explained below, that a considerable amount of IV had formed in a specimen of II kept at room temperature for 16 days and that II is, however, stable for at least 22 months at −15° to −20°.

Since IV has the same composition as II, elemental analyses are of value only for indicating gross contamination; however, infrared spectra of II and its potential contaminants constitute a reliable guide to the quality of the triazene product. If unreacted I is present, it can be detected by the diazo absorption band (which is very strong in pure specimens of I) at 2190 cm.$^{-1}$ (5). If the diazo band is absent but absorption is evident near 1700 cm.$^{-1}$, the product may contain 2-azahypoxanthine, a potential by-product that may be formed either directly from I (5) or from II by a light-catalyzed reaction (6). The most prominent differences between the infrared spectra of II (Fig. 1) and its transformation product (IV, Fig. 2) are as follows: the spectrum of IV shows strong bands at 1515, 1680 (C=O), and 3455–3460 (NH) cm.$^{-1}$; the spectrum of II has strong bands at 1655 (C=O) and 3380 (NH) cm.$^{-1}$. Other obvious differences are shifts of 10–15 cm.$^{-1}$ in the positions of 2 bands in the 1600–1540 cm.$^{-1}$ region, a medium band present at 742 cm.$^{-1}$ in the spectrum of II and absent from that of IV, a medium-strong doublet at 1145 and 1138 cm.$^{-1}$ in the spectrum of II, and a medium band at 768 cm.$^{-1}$ in the spectrum of IV.[1] Of these bands the first listed (1515 cm.$^{-1}$) is the most important for estimating the quality of II. Spectra of the best samples of II show very little, or no, absorption at 1510–1520 cm.$^{-1}$ (Fig. 1). In the spectra of specimens judged to contain only a very few percent of the transformation product, the 1515 cm.$^{-1}$ band can be observed as a very weak band or shoulder, whereas the 1680 cm.$^{-1}$ and 3460 cm.$^{-1}$ bands

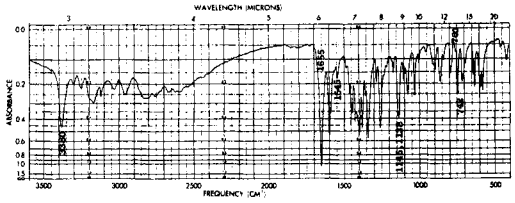

Fig. 1—Infrared spectrum of 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (II). Flattening of the curve in the 1520–1500 cm.$^{-1}$ region may indicate slight contamination with IV.

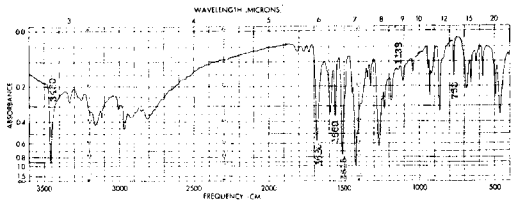

Fig. 2—Infrared spectrum of the transformation product (IV).

[1] However, weak bands at 1138 and 760 cm.$^{-1}$ in the spectra of IV and II, respectively, decrease the value of bands in these regions for detecting either IV or II in samples of the other.

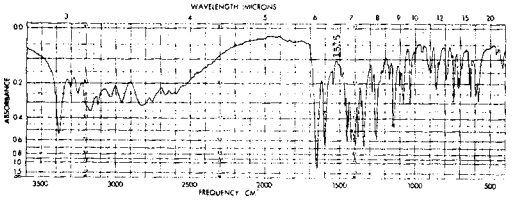

Fig. 3—Infrared spectrum of a specimen of II typical of those used for biological evaluation. The shoulder at 1515 cm.$^{-1}$ shows that a small amount of IV is present.

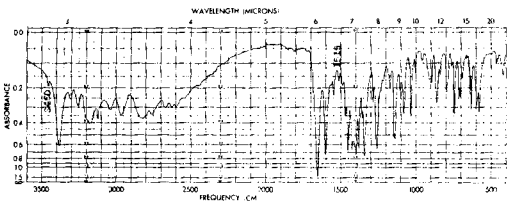

Fig. 4—Infrared spectrum of II showing further contamination with IV. The 3460 cm.$^{-1}$ band of IV appears after the 1515 cm.$^{-1}$ can be detected (cf. *Fig. 3*) *and before the carbonyl band of IV is perceptible. In this spectrum the 1545 cm.$^{-1}$ band of II has also begun to shift to the 1560 cm.$^{-1}$ band of IV.*

are not apparent. Most of the antitumor tests have been performed with samples of this type (Fig. 3). With increasing contamination, the 3460 cm.$^{-1}$ band appears on the side of the 3380 cm.$^{-1}$ band (Fig. 4). Shifting of the band near 1550 cm.$^{-1}$ and splitting of the carbonyl band are readily apparent only when considerable amounts of the transformation product are present.

Infrared spectra of crude products obtained from the ethyl acetate–methanol procedure showed that IV was usually present, the diazo compound was frequently present, and 2-azahypoxanthine was probably sometimes present. Brief and careful washing of these crude products (or of any specimens containing IV) with water at pH 7.3–7.5 reduced the amount of IV to a low concentration, as indicated by very slight absorption at 1515 cm.$^{-1}$, and removed the diazo compound (I) and 2-azahypoxanthine. This procedure may also be used, if necessary, to purify II obtained from the improved procedure. However, specimens from the improved procedure have not contained I detectable by infrared spectra, and, generally, IV has either not been detectable or has been present in quantities that produced only a slight shoulder at 1515 cm.$^{-1}$.

Specimens of II are routinely stored in brown jars that are kept in closed jars or desiccators containing a drying agent, and these containers are stored in a freezer at −15° to −20°. When specimens are to be transferred or weighed, the containers are allowed to come to room temperature before a specimen is removed in order to minimize condensation of atmospheric moisture, which might cause the formation of some of the transformation product on the surface. The infrared spectrum of a specimen stored in this way was essentially unchanged after 22 months. Solutions or suspensions for biological evaluation are prepared immediately before injection and are injected within 5 min., or less, after their preparation.

EXPERIMENTAL

Infrared spectra were recorded with a Perkin-Elmer model 521 spectrophotometer using samples in potassium bromide disks. Ultraviolet spectra were recorded with a Cary model 14 recording spectrophotometer.

5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (II)—*A. Preparation*—Compound II is protected, insofar as possible, from light and moisture during all operations involved in the preparation, handling, and storage. The starting material (I) is also protected from light and moisture (5).

A solution consisting of 625 Gm. of sodium chloride, 2.5 L. of distilled water, and 290 Gm., of bis(2-chloroethyl)amine hydrochloride in a 5-L. Morton flask was chilled in a salt–ice bath. Dichloromethane (1.25 L.) was added, and the mixture was stirred vigorously with a mechanical stirrer while a 50% aqueous sodium hydroxide solution was added until the pH of the mixture reached and became stable at 7.5–7.6. The sodium hydroxide solution was added at a rate that kept the temperature of the mixture below 4°. The mixture was stirred vigorously for 5–10 min., the dichloromethane layer was separated, and the water layer was extracted at the ice-bath temperature with a second 1.25-L. portion of dichloromethane. The two cold dichloromethane extracts were combined, washed quickly with 1 L. of cold saturated salt solution, and given a preliminary drying by stirring with magnesium sulfate in an ice bath for 15 min. The desiccant was removed by filtration, and the solution was dried further by stirring with calcium sulfate[2] for 1 hr. in an ice bath. Smaller additions of desiccant were made at 15-min. intervals. After the drying agent had been removed by filtration, the solvent was evaporated immediately under reduced pressure at 25–30°.

A solution of the bis(2-chloroethyl)amine free base in 1.25 L. of anhydrous methanol (which had been dried 24 hr. over calcium sulfate) was cooled to 15° in a 3-L. round-bottom flask wrapped with aluminum foil and equipped with a mechanical stirrer, a nitrogen inlet tube, and a drying tube. 5-Diazoimidazole-4-carboxamide (49.4 Gm.) was added slowly in small portions in a dry nitrogen atmosphere during 30 min., and the suspension was stirred vigorously for 3 hr. at 15–20°. The product was collected by

[2] Drierite.

filtration under a current of nitrogen, washed under nitrogen with 100 ml. of methanol and with two 100-ml. portions of acetone, and dried *in vacuo* at room temperature over phosphorous pentoxide: yield, 71 Gm. (71%). The absence of discernible absorption bands at 1515 and 2190 cm.$^{-1}$ indicated that the product contained very little, if any, transformation product (IV) or starting material (I).

Anal.—Calcd. for $C_8H_{12}Cl_2N_6O$:[3] C, 34.42; H, 4.33; N, 30.10 Found: C, 34.45; H, 4.30; N, 30.14.

The yields obtained from several experiments in which either dichloromethane or ethyl acetate was used to extract III were 65–75%. Usually absorption due to IV at 1515 cm.$^{-1}$ either was not observed or was very weak.

B. Purification—Specimens of II containing IV, the starting material (I), or 2-azahypoxanthine may be purified by washing the impure material with water adjusted to pH 7.5 with sodium hydroxide, provided that care is exercised to minimize the formation of additional IV. A mixture of the slightly basic solution and II (approximately 10 ml./Gm.) is stirred for 5–10 min. in the absence of light, and the triazene is then removed by filtration, washed with water and with 2 portions of acetone, and dried *in vacuo* over phosphorous pentoxide at room temperature. The purpose of washing with acetone is to hasten the removal of water, but most of the adhering water should be removed during the filtration because II appears to be more soluble in a mixture of acetone and water than in either solvent alone. Since the triazene is only slightly soluble in water, it is recovered in high yield from the washing operation.

Preparation of IV—A mixture of 2.5 Gm. of the triazene(II) and 250 ml. of absolute methanol was stirred in the dark for 44 hr. The mixture became homogeneous, and thin-layer chromatography (silica gel, chloroform–methanol, 1:1 by vol.) indicated that only a trace amount of II remained after 24 hr. (In this TLC system IV remains at the origin; II moves, but shows streaking owing to the formation of IV.) Although the total product from an earlier similar experiment gave satisfactory analytical data without recrystallization (1), the solution was treated with activated carbon, concentrated to about 125 ml., and diluted with an equal volume of chloroform. The yellow precipitate was recrystallized a second time from chloroform–methanol; it melted with decomposition at temperatures that varied with the determination procedure, *e.g.*, 197–199° dec., when inserted at 180° in a Mel-Temp apparatus.

Anal.—Calcd. for $C_8H_{12}Cl_2N_6O$: C, 34.42; H, 4.32; N, 30.10; total Cl, 25.4; ionic Cl, 12.7. Found: C, 34.41; H, 4.44; N, 30.02; total Cl, 25.3; ionic Cl, 12.6.

[3] Analysis of the product of an earlier experiment gave a satisfactory value for chlorine (Calcd.: 25.5%, found: 25.4%), as well as satisfactory data for carbon, hydrogen, and nitrogen (1).

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Keyphrases

Antileukemic agent
5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]-imidazole-4(or 5)-carboxamide,—syntheses
Triazene transformation product
TLC separation
IR spectrophotometry—structure
UV spectrophotometry—structure