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Synthesis, Antileukemic Activity, and Stability of 3-(Substituted-Triazeno)pyrazole-4-carboxylic Acid Esters and 3-(Substituted-Triazeno)pyrazole-4-carboxamides

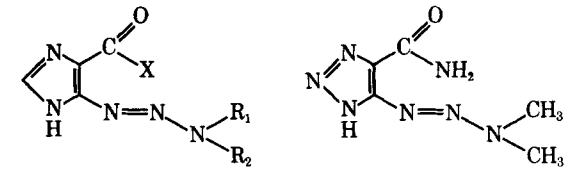
Y. FULMER SHEALY and C. ALLEN O'DELL

Abstract □ Representative 3-triazeno derivatives of the ethyl and methyl esters of pyrazole-4-carboxylic acid and of pyrazole-4-carboxamide were synthesized from the corresponding 3-diazopyrazoles. It was shown that certain triazenopyrazoles in solution are decomposed by light, and transformations of 3-(3-methyl-1-triazeno)pyrazole-4-carboxamide and 3-[3,3-bis(2-chloroethyl)-1-triazeno]pyrazole-4-carboxamide in solution in the dark to 3-aminopyrazole-4-carboxamide and to a *v*-triazolinium salt, respectively, were observed. All of these transformations are analogous to reactions of triazenoimidazoles observed previously. Triazenopyrazoles having both the ester and the amide groups increased the average survival time in the standard mouse L-1210 leukemia assay. In some of these tests, increases in lifespan of 60–120% were observed.

Keyphrases □ Triazenopyrazoles—synthesis, decomposition by light □ Leukemia L-1210 inhibition—triazenopyrazoles □ Antileukemic activity—triazeno derivatives of pyrazole esters, amides □ Diazopyrazole esters—antimicrobial activity

Various triazenoimidazolecarboxamides (*Ia*) (1–5) and triazenoimidazolecarboxylates (*Ib*) (6, 7) have been synthesized, and a number of these have displayed antineoplastic activity (2, 3, 5–10). Several triazeno-*v*-triazoles (*e.g.*, II) were prepared as ring analogs of the imidazoles, and activity was also found among derivatives of this ring system (11). It seemed logical, therefore, to extend these studies to related heterocycles. The synthesis and antileukemic activity of 3-(substituted-triazeno)pyrazole-4-carboxylic acid esters (V–VIII) and 3-(substituted-triazeno)pyrazole-4-carboxamides (X–XIV) are described in the present report. The bis(2-fluoroethyl)triazenopyrazoles (IX and XV) comprised part of a recent communication (12); after most of this work had been completed, one of the other derivatives, the dimethyltriazeno derivative (X) of the amide series, was reported by Noell and Cheng (13). The dimethyltriazeno derivative of antipyrine, a pyrazolone, was prepared by Stolz (14) in 1908.

The triazenopyrazoles (V–XV) were prepared by the methods utilized for the synthesis of triazenoimidazoles (I), namely, by isolating a diazopyrazole and treating it in nonaqueous media with the appropriate amine or by diazotizing the aminopyrazole and then adding the amine to the aqueous solution without isolating the intermediate diazo derivative. Both methods afforded the simple dialkyltriazenopyrazolecarboxylate esters (V–VIII). The first method was used in syntheses, or attempted syntheses, of unstable triazenes, such as bis(2-haloethyl)triazeno derivatives and the monomethyltriazene (XIII); it is preferable for the preparation of triazenopyrazolecarboxamides because of the competing intramolecular cyclization reaction to 3,7-

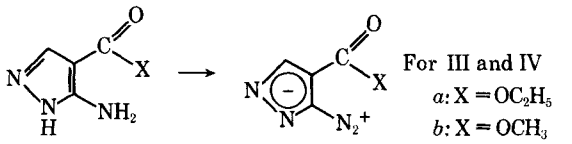


I

a: X = NH_2

b: X = OR

II



III IV

For III and IV

a: X = OC_2H_5

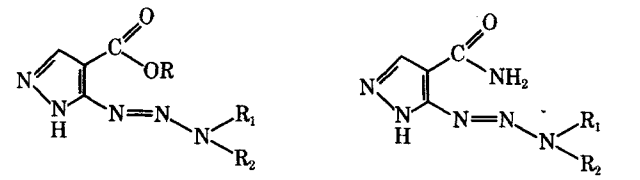
b: X = OCH_3

c: X = NH_2

Scheme I

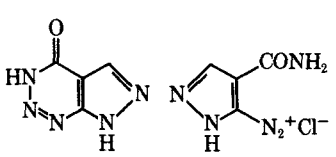
dihydro-4*H*-pyrazolo[3,4-*d*]-*v*-triazin-4-one (XVI) (15). Although 3-(3-butyl-3-methyl-1-triazeno)pyrazole-4-carboxamide (XI) was obtained without isolating 3-diazopyrazole-4-carboxamide (IV*c*), the dimethyltriazeno derivative (X) was more readily obtained pure (86% yield) by using the anhydrous medium (ethyl acetate) employed in some of the triazenoimidazole syntheses (5). This method was also used in the previously reported synthesis (13). The two bis(2-fluoroethyl)triazeno derivatives (IX and XV) were isolated and purified without causing serious difficulties (12), but 3-[3,3-bis(2-chloroethyl)-1-triazeno]pyrazole-4-carboxamide (XIV), like the analogous imidazole (4, 10), is less stable and more difficult to isolate. A specimen was eventually obtained that, according to NMR assay, contained only about 3% of the *v*-triazolinium salt formed by intramolecular cyclization. (See *References 4* and *16* for characterization of the analogous imidazole.)

Both ethyl and methyl 3-diazopyrazole-4-carboxylates (IV*a* and IV*b*) were isolated as pure crystalline compounds from small-scale diazotization reactions either by adding the aminopyrazoles (III*a* and III*b*) to aqueous sodium nitrite or by employing the reverse mode of addition (Scheme I). A diazotization on a modestly increased scale performed by adding the nitrite solution to III*b* yielded principally the bis(pyrazolyl)triazene (XVIII). This compound was then deliberately prepared by using an excess of III*b*. Employment of III*c* free base and only one equivalent of hydrochloric acid resulted in the precipitation of the diazo derivative (IV*c*). However, the procedure (13) of diazotizing the aminopyrazole hemisulfate, without added acid, is preferable because preparation of the free base is not required. When excess hydrochloric acid was used in diazotizing III*c* sulfate or free base, attempts to isolate the diazo amide (IV*c*) or to form a triazene *in situ* usually gave mixtures of the desired product and the pyrazolo-*v*-triazinone (XVI). As already intimated, the isolation of pure XI was an exception. A possible explanation for the adverse effect of excess acid on the isolation of IV*c* is that the latter compound is protonated to the diazonium chloride (XVII), which should be soluble and should cyclize readily to XVI.



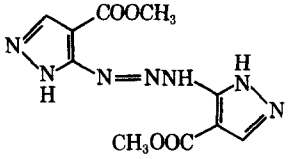
R	R_1	R_2
V: C_2H_5	CH_3	CH_3
VI: CH_3	CH_3	CH_3
VII: C_2H_5	CH_3	*n*-C_4H_9
VIII: CH_3	CH_3	*n*-C_4H_9
IX: C_2H_5	$-CH_2CH_2F$	$-CH_2CH_2F$

	R_1	R_2
X:	CH_3	CH_3
XI:	CH_3	*n*-C_4H_9
XII:	CH_3	$-CH_2CH_2OH$
XIII:	CH_3	H
XIV:	$-CH_2CH_2Cl$	$-CH_2CH_2Cl$
XV:	$-CH_2CH_2F$	$-CH_2CH_2F$



XVI

XVII



XVIII

The monomethyltriazeno derivative (XIII), like the analogous imidazole (2), decomposed spontaneously in the dark in aqueous methanol to 3-aminopyrazole-4-carboxamide (III*c*).

This study included observations on the stability of 3-(3,3-dimethyl-1-triazeno)pyrazole-4-carboxamide (X) and of the methyl ester (VI) in comparison with the analogous triazenoimidazoles. These observations differ considerably from previous statements relating to the stability of X; it was reported (13) that X is unchanged during 3 days of "exposure to daylight" in aqueous buffer at pH 7, and the implied stability to light was contrasted with the behavior of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, which is known to be dissociated in solution under certain conditions of light exposure and to remain unchanged during several days under other conditions (1, 15, 17). For this study, solutions of X and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide contained in Pyrex volumetric flasks placed side-by-side were exposed simultaneously[1], and changes were observed by determining the UV spectra of aliquots withdrawn from the two solutions. 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide and its pyrazole analog (X) were exposed in this way to light as follows: (*a*) in 50% aqueous ethanol in direct sunlight (filtered through window glass); (*b*) in 50% aqueous ethanol at a distance of 10 cm. from a UV light source that emits radiation principally at 365 nm.; and (*c*) in a Krebs–Ringer phosphate buffer (18) at a distance of 15 cm. from the same UV source. Two generalizations summarize the results: (*a*) in all three experiments, both the triazenopyrazole (X) and the triazenoimidazole [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide] decomposed, as evidenced by the decrease or disappearance of their long wavelength absorption

[1] Comparisons of the relative stabilities of light-sensitive compounds (or of the rates of change of a given compound in different solutions) are valid only if the conditions of exposure are identical. The wavelength and intensity of sunlight reaching exposed compounds are determined by a number of variables such as latitude, time of the day, time of the year, transmission properties of the glass through which the light passes, and the clarity of the atmosphere. When two solutions are placed side-by-side, these conditions are equalized, but they may not be reproducible.

Table I—Triazenopyrazoles and Diazopyrazoles *versus* Leukemia L-1210[a]

Compound	Dose[b], mg./kg., and Schedule[c]	Mortality, Deaths/Total	Average Weight Change, g., T/C	Average Survival Time	
				T/C, Days	T/C Ratio[d], %
V	400 D2	0/6	−1.2/+2.6	10.3/8.0	128
	200 D2	0/6	−0.6/+2.6	10.0/8.0	125
	600	0/6	−4.6/+2.4	12.0/9.3	129t
	400 D2	0/6	−1.3/+2.4	13.2/9.3	141
	267 DR	0/6	−0.7/+2.4	10.5/9.3	112
	178	0/6	+0.8/+2.4	10.3/9.3	110
	200	2/6	−3.5/+1.8	12.3/9.1	135
	120 q.d. 1–9	0/6	−1.7/+1.8	12.2/9.1	134
	72 DR	0/6	−1.0/+1.8	11.5/9.1	126
	42	0/6	−0.9/+1.8	10.3/9.1	113
VI	400 D2	0/4	−3.0/+2.4	12.0/9.8	122t
	200 DR	0/4	−1.9/+2.4	11.8/9.8	120t
	100	0/4	−0.5/+2.4	10.5/9.8	107
	400 q.d. 1–9	2/6	−5.2/+1.1	7.0/9.0	77t
	200 q.d. 1–9	0/12	−3.3/+1.4	14.6/8.9	164[e]
	300	1/6	−5.3/+1.1	7.2/8.1	88
	200 q.d. 1–9	0/6	−3.7/+1.1	10.5/8.1	129
	133 DR	0/6	−2.8/+1.1	13.3/8.1	164
	88	0/6	−2.1/+1.1	14.2/8.1	175
	88	0/5	−1.5/+0.9	13.6/8.3	163
	58 q.d. 1–9	0/6	−1.8/+0.9	13.0/8.3	156
	38 DR	0/6	−1.3/+0.9	11.3/8.3	136
	25	0/6	−0.5/+0.9	10.0/8.3	120
VII	400 D1	0/6	−0.5/+1.9	10.3/9.0	114
	400 q.d. 1–9	0/13	−1.5/+1.1	13.8/8.5	162[e]
	600	3/6			t
	400 q.d. 1–9	0/6	−2.9/+1.6	11.5/8.9	129
	266 DR	0/6	−2.1/+1.6	15.0/8.9	168
	177	0/6	−1.3/+1.6	12.3/8.9	138
VIII	400 D1	0/6	+0.3/+1.9	10.0/9.0	111
	400 q.d. 1–9	0/6	−3.6/+0.7	14.0/8.7	160
	600	1/6	−2.6/+1.5	8.0/9.0	88
	400 q.d. 1–7	0/6	−2.2/+1.5	12.3/9.0	136
	268 DR	0/6	−1.8/+1.5	13.5/9.0	150
	176	0/6	−1.4/+1.5	11.8/9.0	131
X[f]	400 D1	0/6	−2.0/+2.5	12.0/8.5	141t
	300	0/6	−2.0/+1.0	12.0/8.3	144
	150 D1	0/6	−1.4/+1.0	10.2/8.3	122
	75 DR	0/6	−0.6/+1.0	8.3/8.3	100
XI	400 D1	0/6	−3.6/−0.3	9.2/8.6	106
	200 D1	1/6	−1.5/+1.7	10.6/9.2	115
	400 q.d. 1–9	7/7			t
	100 q.d. 1–9	0/12	−2.5/+1.8	12.3/8.5	145[e]
	150	0/6	−3.7/+1.4	9.5/8.3	114
	100 q.d. 1–9	0/6	−2.9/+1.4	11.7/8.3	140
	66 DR	0/6	−1.6/+1.4	15.3/8.3	184
	44	0/6	−1.0/+1.4	13.7/8.3	165
XII	400 D1	0/6	+2.1/+2.6	9.7/8.8	110
	400 q.d. 1–9	0/12	−1.2/+1.4	14.9/8.9	167[e]
	600	0/6	−1.2/+3.0	16.7/7.5	222
	400 q.d. 1–9	0/6	−1.5/+3.0	15.5/7.5	205
	268 DR	0/6	−0.2/+3.0	12.7/7.5	169
	176	0/6	+1.2/+3.0	11.2/7.5	149
	176 q.d. 1–9	0/6	−0.6/+1.6	13.2/8.3	159
	118 DR	0/6	−0.5/+1.6	11.2/8.3	134
	80	0/6	−0.2/+1.6	10.8/8.3	130
XIV[g]	300	3/6	−0.3/+1.2	11.7/9.3	125t
	150 D1	0/5	+0.7/+1.2	10.4/9.3	111
	75 DR	0/6	+1.7/+1.2	9.7/9.3	104
	150	0/6	+0.6/+1.7	12.3/8.3	148
	100 q.d. 1–9[h]	0/6	+0.6/+1.7	12.0/8.3	144
	67 DR	0/6	+0.5/+1.7	10.2/8.3	122
IV*a*	40 D1	6/6			t
	20 D1	0/6	−1.1/+2.0	9.2/9.0	102
	10 D1	0/6	+0.8/+2.0	8.7/9.0	96
	20 q.d. 1–9	6/6			t
	10 q.d. 1–9	0/6	−1.9/+1.9	7.5/8.8	85t
	5 q.d. 1–9	0/6	−0.6/+0.7	9.0/8.4	107

Table I—*Continued*

Compound	Dose[b], mg./kg., and Schedule[c]	Mortality, Deaths/Total	Average Weight Change, g., T/C	Average Survival Time— T/C, Days	T/C Ratio[d], %
IV*b*	40 D1	6/6			t
	20 D1	0/6	−0.8/+2.0	8.8/9.0	97
	20 q.d. 1–9	6/6			t
	10 q.d. 1–9	1/6	−1.9/+1.9	7.2/8.8	81t
	5 q.d. 1–9	0/6	−1.1/+0.7	9.2/8.4	109
XVIII	400 D1	0/6	+0.9/+2.8	7.5/8.7	86
	400 q.d. 1–9	0/6	−2.4/+1.0	8.0/9.2	86

[a] Solutions or suspensions of the compounds (VI, IV*a*, and IV*b* in physiological saline, V in physiological saline containing 0.4% carboxymethylcellulose, and the remaining compounds in physiological saline containing 0.15% polysorbate 80) were protected from light and were injected immediately after they were prepared. Mice were implanted intraperitoneally on Day 0 with 10^5 L-1210 cells. Treatment schedules designated D1 (Day 1) or D2 (Day 2) mean that a single dose was injected intraperitoneally on the 1st day (about 24 hr.) or on the 2nd day (about 48 hr.), respectively, after implantation; q.d. 1–9 means that daily injections at the specified dose were initiated on Day 1 and continued through Day 9 or until the death of the animal. Average weight change = average weight change of host animals. Mortality and weight changes of host animals were determined 4 days after the first (for daily treatment) or only (for Day 1 or Day 2) injection. T = treated mice, C = control (untreated leukemic) mice. [b] Some of the higher (toxic) and lower (inactive) doses are not included. [c] DR = dose response. These doses were administered on the designated schedule on the same day in the same controlled experiment. [d] t = toxic. A rating of toxic may be based upon mortality (>2/6), a value of T/C of 85% or less, or a weight-change difference exceeding −4 g. after Day 1 or Day 2 treatment. The latter criterion is not applied to results of daily administration. [e] Values in the last three columns are approximate averages of two tests. [f] *cf.*, *Reference 13* for additional data. [g] NMR assays showed that the specimen used for daily treatment contained about 25% of the isomeric *v*-triazolinium salt at the beginning of the tests; the specimen used for the D1 tests was similarly shown to contain about 3–4% of the *v*-triazolinium salt. [h] The dose of 150 mg./kg./day was administered q.d. 1–8.

maxima; and (*b*) the triazenoimidazole disappeared more rapidly than did its pyrazole analog.

Solutions of the pyrazole methyl ester (VI) and the analogous imidazole methyl ester (*Ib*, R = R_1 = R_2 = CH_3) (6) in 50% aqueous ethanol were exposed simultaneously in the same way to 365-nm. radiation at a distance of 10 cm. Again, both compounds decomposed, and the imidazole derivative decomposed significantly faster than the pyrazole derivative. The following two comparisons also appear to be justified, but there is less assurance that the experimental conditions for the photochemical change were equalized: (*a*) although the two methyl esters were not exposed simultaneously with their amide analogs, the last-mentioned experiment was comparable to Experiment *b* with the two amides, and the results indicate that the esters change more slowly in 365-nm. light than do their amide analogs; and (*b*) exposure of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and its *v*-triazole analog similarly, but not simultaneously, in 50% methanol to 365-nm. radiation resulted, in agreement with an earlier suggestion (11), in slower disappearance of the triazeno-*v*-triazole. Consistent with, but not directly comparable to, the foregoing observations of light sensitivity of triazenopyrazoles was an earlier observation of slow decomposition of XI in absolute methanol under ambient lighting conditions. Although the triazenopyrazoles may not be rapidly degraded under conditions of normal use, the observations described here show that they should be handled as light-sensitive compounds until their stability to prevailing lighting conditions is determined.

BIOLOGICAL ACTIVITY

Results of tests of Compounds V–VIII, X–XIV, and XVIII *versus* murine lymphoid leukemia L-1210 are summarized in Table I. The data show that triazeno derivatives of both the ester and amide series prolong the survival time of treated animals. Increases in survival time of 60–122% (T/C = 160–222%) were recorded in some tests of the esters VI–VIII and of the amides XI–XII administered in multiple daily doses (q.d. 1–9), and the dose–response data for these derivatives are internally consistent. Compounds V, X, and XIV also significantly increased the lifespan of treated mice. The 3-(2-hydroxyethyl)-3-methyl-1-triazeno derivative (XII) resembles three 5-[3-(2-hydroxyethyl)-3-methyl-1-triazeno]imidazoles (5, 7) in inhibiting L-1210 at doses higher than tolerated doses of similar dialkyltriazeno derivatives. The three 3,3-[bis(2-haloethyl)-1-triazeno]pyrazoles (IX, XIV, and XV) were less effective in these initial screening tests than were the corresponding imidazole derivatives (Table I) (10, 12). The fact that the activity of XIV is less than that of the analogous imidazole (NSC-82196) may be the result of a faster rate of transformation to the isomeric *v*-triazolinium salt, but there are no data to support this supposition. The bulk material used in the single-dose tests was at least comparable in quality to specimens of NSC-82196 used in similar tests; the specimen used for chronic therapy contained about 25% of the *v*-triazolinium salt.

In tests *in vitro* of Compounds V–XV and XVIII in the paper-disk-agar-plate method, there was no observable inhibition of several Gram-negative and Gram-positive bacteria or of several species of fungi, except for evidence of slight inhibition of *Streptococcus faecalis* (ATCC 8043) by Compounds V, VI, and IX at 100 mcg./disk. The absence of antimicrobial activity contrasts with the antimicrobial activity of triazenoimidazoles, particularly with the broad-spectrum activity of the esters (6, 7, 19). The diazopyrazole esters (V and VI), but not the amide (X), displayed antibacterial and antifungal activity *in vitro*.

EXPERIMENTAL

During all operations employed in the preparation, isolation, or purification of the triazenopyrazoles, they were routinely protected from light. Isolated specimens were routinely stored in a refrigerator, except for bis(2-haloethyl)triazeno derivatives which were stored in a freezer at −10 to −15°. UV and IR spectra were recorded with a Cary spectrophotometer (model 14) and with Perkin-Elmer spectrophotometers (models 521 or 621), respectively. The IR bands listed were taken from spectra of samples in pressed KBr disks, unless stated otherwise, and are in $cm.^{-1}$; s = strong, m = medium, w = weak, b = broad, and sh = shoulder. Solutions used to determine UV data were prepared by diluting a 5-ml. aliquot of an ethanol solution of the compound to 50 ml. with 0.1 *N* HCl, phosphate buffer (pH 7), or 0.1 *N* NaOH; absorption maxima are in nanometers.

Melting or decomposition temperatures were determined in a capillary tube with a Mel-Temp or a Mettler FP-1 apparatus unless otherwise stated. TLC was performed by applying 20- and 40-mcg. portions of a compound in ethanol (IV*a*, IV*b*, VI–VIII, and X–XII) or methanol (IX, XIV, XV, and XVIII) to silica gel plates. The developing solvents are given in the experimental procedures. The methods of detection of spots on all chromatograms were both UV absorption (254 and 365 nm.) and UV light (254 nm.) after spraying with an optical whitening agent[2]. In addition, some chromatograms were also examined after exposure to iodine vapor.

[2] Ultraphor WT, BASF Colors and Chemicals, Inc., Charlotte, N. C.

Ethyl 3-Diazopyrazole-4-carboxylate (IV*a*)—*Procedure A*—To a solution of 10 mmoles (1.55 g.) of ethyl 3-aminopyrazole-4-carboxylate (III*a*) in 25 ml. of 1 *N* HCl at 0° was added, during 5 min., a solution of 11 mmoles (760 mg.) of sodium nitrite in 5 ml. of water. The resulting turbid solution was stirred at 0° for 0.5 hr. and filtered, the filtrate was extracted with three 50-ml. portions of chloroform, and the chloroform layers were combined. The total chloroform extract was washed with 50 ml. of saturated NaCl solution, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The residual yellow syrup crystallized: yield, 849 mg. (51%); m.p. 67–68° (Kofler Heizbank); TLC, 1 spot (9:1 $CHCl_3$–CH_3OH); IR bands at 2290w, 2110w, 2210s (N_2^+), 1700s (ester CO), 1530ms, and 768 ms.

Anal.—Calcd. for $C_6H_6N_4O_2$: C, 43.38; H, 3.64; N, 33.72. Found: C, 43.29; H, 3.54; N, 33.67.

Procedure B—This procedure was the same as Procedure A except that: (*a*) the solution of III*a* was added to the solution of sodium nitrite at such a rate that the temperature did not exceed 3°, and (*b*) the reaction mixture was stirred for 1 hr. prior to the chloroform extraction. The yield of IV*a*, identical with a specimen obtained by Procedure A, was 44%.

Methyl 3-Diazopyrazole-4-carboxylate (IV*b*)—This compound was prepared from 10 mmoles of III*b* by a procedure that otherwise duplicated Procedure A for the preparation of IV*a*: yield, 21%; m.p. 72–73°; TLC, 1 spot (9:1 $CHCl_3$–CH_3OH); IR bands at 2285w, 2120w, 2215s (N_2^+), 1710s (ester CO), 1535ms, 1525sh, and 760ms.

Anal.—Calcd. for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.84. Found: C, 39.24; H, 2.69; N, 36.71.

A similar diazotization of 8 g. of III*b*, performed for the purpose of preparing VIII without isolating IV*b*, gave chiefly the bis(pyrazolyl)triazene (XVIII) (see the procedures for VIII). Addition of the solution of III*b* (8 g. in 100 ml. of 1 *N* HCl) to the nitrite solution (4.31 g. in 50 ml. of water) during 40 min. at 0–3°, followed by stirring for 0.5 hr. and isolation as in Procedure A for IV*a*, gave IV*b* in 41% yield (m.p. 72–73°). Recrystallization of a specimen by diluting an ethyl acetate solution (previously treated with activated carbon and concentrated *in vacuo*) with cyclohexane gave pale-yellow crystals which were apparently in a different crystal form: m.p. 91–92°; TLC, 1 spot (98:2 $CHCl_3$–CH_3OH); IR spectrum, identical with that of the specimen obtained by the first procedure except for some differences in the 1300–850 $cm.^{-1}$ region.

Anal.—Calcd. for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.84. Found: C, 39.39; H, 2.60; N, 36.82.

3-Diazopyrazole-4-carboxamide (IV*c*)—This compound was prepared by Procedure A for IV*a* with the following exceptions: (*a*) equimolar amounts of III*c* (free base) and 1 *N* HCl were used, and (*b*) the product was separated by filtration after 2.5 hr. of stirring at 0°. The yellow solid (42% yield) exploded on a Kofler Heizbank apparatus at about 180–185°. The IR spectrum was identical with that of a specimen prepared by the procedure subsequently reported by Cheng *et al.* (15): IR bands at 2215sb (N_2^+), 1680s, 1625s, 1535s, 775mw, 750mw, and 722s.

Ethyl 3-(3,3-Dimethyl-1-triazeno)pyrazole-4-carboxylate (V)—Procedure A for the preparation of IV*a* was duplicated with the following exceptions: (*a*) the reaction time was 1 hr.; (*b*) isolation of IV*a* by chloroform extraction was omitted; and (*c*) excess nitrosating agents were decomposed with sulfamic acid. The cold aqueous solution of IV*a* was diluted with 15 ml. of 25% aqueous dimethylamine, and the resulting solution was stirred at room temperature for 2.5 hr. Extraction of the reaction mixture with chloroform as described in Procedure A for IV*a* afforded a pale-yellow crystalline solid (1.9 g., 90%). Dissolution of the crude triazene in 20 ml. of hot ethyl acetate, treatment of the solution with activated carbon, dilution of the hot filtrate with 20 ml. of cyclohexane, and cooling the resulting solution afforded yellow platelets: wt., 1.4 g. (71%); m.p. 113.5–115.5°; IR bands at 1710s and 1685s (ester CO), 1570m, 1495s, and 775m. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 226 (9.7) and 312 (12.1) in 0.1 *N* HCl; 230 (11.0) and 313 (11.2) at pH 7; 244 (14.2) and 314 (8.8) in 0.1 *N* NaOH.

Anal.—Calcd. for $C_8H_{13}N_5O_2$: C, 45.49; H, 6.20; N, 33.16. Found: C, 45.64; H, 6.19; N, 32.89.

Methyl 3-(3,3-Dimethyl-1-triazeno)pyrazole-4-carboxylate (VI)—*Procedure A*—By a procedure similar to that described for the preparation of V, Compound VI was prepared from 2 g. of III*b*. A small quantity of an unidentified solid was filtered from the reaction mixture before the extraction with chloroform. Recrystallization of crude VI from ethyl acetate–cyclohexane (1:3) in the manner described for V gave white crystals: yield, 62%; m.p. 103°; TLC, 1 spot (95:5 $CHCl_3$–CH_3OH); IR bands at 1720s (ester CO), 1568ms, 1485ms, and 772m. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 226 (9.5) and 312 (12.1) in 0.1 *N* HCl; 230 (10.9) and 313 (11.3) at pH 7; 244 (13.7) and 315 (8.95) in 0.1 *N* NaOH.

Anal.—Calcd. for $C_7H_{11}N_5O_2$: C, 42.63; H, 5.62; N, 35.52. Found: C, 42.41; H, 5.64; N, 35.65.

Procedure B—Anhydrous dimethylamine was bubbled into 35 ml. of ethyl acetate at 0–5° until the volume had increased to about 40 ml., and 1.8 g. of IV*b* was added in small portions during 1 hr. at 0°. The mixture was stirred for an additional hour at 0°, treated with activated carbon, and concentrated *in vacuo* to a yellow solid which was triturated with a mixture (9:1) of cyclohexane–ethyl acetate. Recrystallization of the residual solid from ethyl acetate–cyclohexane gave 1.22 g. of VI (m.p. 103–104°) identical with the specimen obtained from Procedure A.

Ethyl 3-(3-Butyl-3-methyl-1-triazeno)pyrazole-4-carboxylate (VII)—To a solution of 1.96 g. of sodium nitrite in 10 ml. of water at 0° was added dropwise a cold solution of 4.0 g. of III*a* in 50 ml. of 1 *N* HCl at a rate that kept the temperature at 0–5°. The yellow solution was stirred at 0° for 1 hr., excess nitrosating agents were destroyed by the addition of sulfamic acid, the mixture was filtered, the filtrate was diluted with 10 ml. of *n*-butylmethylamine, and the resulting reaction mixture was stirred in the dark at room temperature for 3 hr. The chloroform solution obtained by extracting the reaction mixture with three 70-ml. portions of chloroform was washed with 70 ml. of saturated aqueous NaCl, dried with $MgSO_4$, treated with activated carbon, filtered through diatomaceous earth[3], and evaporated *in vacuo*. The residual yellow syrup (6.5 g.) was dissolved in purified cyclohexane and a small quantity of acetone (to effect dissolution) and applied to a column (60 g.) of magnesia-silica gel[4] prewashed with cyclohexane. The column was eluted with cyclohexane and then with acetone, and the fractions shown by TLC to contain pure VII were combined and concentrated *in vacuo*. The residue was a very viscous, faintly yellow syrup which was kept *in vacuo* for a prolonged period to remove the last traces of solvents: yield, 5.7 g. (88%); TLC, 1 spot (95:5 or 99:1 $CHCl_3$–CH_3OH); IR bands (film) at 1715sh and 1690s (ester CO), 1565ms, 1490sh, and 775ms. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 313 (12.3) in 0.1 *N* HCl; 231 (10.6) and 313 (11.4) at pH 7; 245 (14.4) and 313 (8.8) in 0.1 *N* NaOH.

Anal.—Calcd. for $C_{11}H_{19}N_5O_2$: C, 52.16; H, 7.56; N, 27.65. Found: C, 52.33; H, 7.67; N, 27.77.

Methyl 3-(3-Butyl-3-methyl-1-triazeno)pyrazole-4-carboxylate (VIII)—This compound was obtained from III*b* (6.3 g.) by a procedure similar to that described for the preparation of VII. The yellow syrup obtained from the chloroform extract was precipitated from an ether solution by the addition of cyclohexane. This process was repeated, the purified syrup was then treated in ether with activated carbon, and the ether filtrate was concentrated *in vacuo* to a viscous, colorless syrup which was kept *in vacuo* for 16 hr. at room temperature and for 1 hr. at 56°: yield, 59%; TLC, 1 spot (99:1 $CHCl_3$–CH_3OH); IR bands (film) at 1715sh and 1695s (ester CO), 1565ms, 1480sh, and 780m. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 313 (12.2) in 0.1 *N* HCl; 230 (10.6) and 314 (11.7) at pH 7; 244 (14.1) and 314 (9.1) in 0.1 *N* NaOH. When VIII was stored at low temperature, it crystallized to a low melting solid.

Anal.—Calcd. for $C_{10}H_{17}N_5O_2$: C, 50.19; H, 7.16; N, 29.27. Found: C, 50.03; H, 7.28; N, 29.29.

A pure specimen of VIII was initially obtained in 27% yield from 2 g. of III*b* by a similar procedure, except that the diazotization was performed by adding the nitrite solution immediately to III*b*. However, on a somewhat larger scale (8 g. of III*b* in 100 ml. of 1 *N* HCl and 4.32 g. of sodium nitrite in 20 ml. of water), this procedure gave chiefly XVIII (5.3 g.), which was removed by filtration after excess nitrite was decomposed. The filtrate afforded only a slight yield of VIII.

Dimethyl 3,3′-Diazoaminodipyrazole-4-carboxylate (XVIII) (*cf.*, the preceding paragraph)—To a solution of 1.411 g. (10 mmoles) of III*b* in 25 ml. of 1 *N* HCl at 0° was added, over a 5-min. period, a solution of 390 mg. (5.65 mmoles) of sodium nitrite in 5 ml. of water. A heavy yellow precipitate was present in the solution (after 1.5 hr.), but the reaction mixture gave a positive Bratton–Marshall test. An additional 706 mg. (5 mmoles) of III*b* in 10 ml. of water was

[3] Celite, Johns-Manville.
[4] Florisil, The Floridin Co.

added. The mixture was stirred at room temperature for 18 hr., at which time a second solution of 706 mg. of III*b* in 10 ml. of water was added and stirring was continued for 2 hr. The yellow precipitate was collected by filtration, washed thoroughly with water, and dried *in vacuo* at 56°: yield, 1.50 g.; m.p. 153–155° dec. The analytical sample was obtained by recrystallizing 500 mg. of the crude product from 140 ml. of boiling ethanol: recovery of pale-yellow crystals, 350 mg.; m.p. 162–163° dec.; TLC, 1 spot (5:1 $CHCl_3$–CH_3OH or 86:14 butanol–water); IR bands at 1715s and 1690s (ester CO), 1615w, 1580s, 1570sh, 1550ms, 1520m, 1492s, and 778s. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 232 (13.1) and 347 (15.3) at pH 7.

Anal.—Calcd. for $C_{10}H_{11}N_7O_4$: C, 40.95; H, 3.78; N, 33.44. Found: C, 40.75; H, 3.90; N, 33.44.

3-(3,3-Dimethyl-1-triazeno)pyrazole-4-carboxamide (X)—This compound was prepared from IV*c* by a procedure similar to Procedure B for VI, except that the additional stirring time was 2.5 hr. and the precipitated X was isolated by filtration (86% yield) and could be recrystallized from ethyl acetate containing about 2% ethanol: m.p. 188–190° dec. (inserted at 170°, heating rate 3°/min.); TLC, 1 spot (5:1 $CHCl_3$–CH_3OH); IR bands at 1640s, 1595ms, 1500m, 1480m, and 778w. UV extinction coefficients were somewhat higher and the decomposition temperature was somewhat lower than those reported (13). $UV_{max.}$ ($\epsilon \times 10^{-3}$): 317 (14.5) in 0.1 *N* HCl; 230 (11.2) and 318 (13.0) at pH 7; 243 (12.1) and 325 (11.2) in 0.1 *N* NaOH.

Anal.—Calcd. for $C_6H_{10}N_6O$: C, 53.52; H, 5.53; N, 46.13. Found: C, 53.71; H, 5.59; N, 45.95.

3-(3-Butyl-3-methyl-1-triazeno)pyrazole-4-carboxamide (XI)—A solution of IV*c* was prepared by adding slowly a solution of 301 mg. of sodium nitrite in 5 ml. of water to a suspension of 500 mg. of III*c* hemisulfate in 8.3 ml. of 1 *N* HCl at 0°, stirring the mixture for 15 min., destroying excess nitrosating agents with sulfamic acid, and filtering to remove a yellow precipitate. The filtrate was diluted with 3 ml. of *n*-butylmethylamine, the reaction mixture was stirred at room temperature for 1 hr., and the pH of the solution was adjusted to 6.6 with aqueous HCl. Addition of 50 ml. of chloroform caused the separation of platelets which were collected by filtration after the mixture had been chilled: yield, 550 mg. (62%); m.p. 142–145° dec. Recrystallization of 450 mg. of the crude product from ethyl acetate–cyclohexane (3:5) in the manner described for V gave white crystals: recovery, 325 mg.; m.p. 148–150° dec.; TLC, 1 spot (9:1 $CHCl_3$–CH_3OH); IR bands at 1645s, 1595s, 1505ms, 810mb, and 790sh. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 319 (16.2) in 0.1 *N* HCl; 232 (11.3) and 319 (14.3) at pH 7; 244 (12.3) and 325 (12.6) in 0.1 *N* NaOH. The extinction coefficient (14.7) at 320 nm. of a methanol solution of XI left unprotected from light decreased by about 10% during 48 hr.

Anal.—Calcd. for $C_9H_{16}N_6O$: C, 48.20; H, 7.18; N, 37.48. Found: C, 48.25; H, 6.90; N, 37.20.

3-[3-(2-Hydroxyethyl)-3-methyl-1-triazeno]pyrazole-4-carboxamide (XII)—To a solution of 2.06 g. (27.5 mmoles) of freshly distilled 2-(methylamino)ethanol in 25 ml. of anhydrous ethanol at 0° was added, during 0.5 hr., 1.25 g. (9.1 mmoles) of IV*c*; the resulting mixture was stirred at 0° for 0.5 hr. The yellow precipitate was collected by filtration, washed with ethanol, and dried *in vacuo* at 56°: yield, 1.50 g. (78%); m.p. 172–174° dec. Recrystallization of a specimen from ethanol, with the aid of activated carbon, gave white crystals: m.p. 173–175° dec.; TLC, 1 spot (3:1 $CHCl_3$–CH_3OH); IR bands at 1650s, 1615s, 1510ms, 1480ms, and 780m. $UV_{max.}$ ($\epsilon \times 10^{-3}$): 225 (10.1) and 318 (15.2) in 0.1 *N* HCl; 230 (11.4) and 318 (13.6) at pH 7; 243 (12.6) and 325 (12.1) in 0.1 *N* NaOH.

Anal.—Calcd. for $C_7H_{12}N_6O_2$: C, 39.62; H, 5.71; N, 39.61. Found: C, 39.72; H, 5.37; N, 39.25.

3-(3-Methyl-1-triazeno)pyrazole-4-carboxamide (XIII)—The monomethyltriazeno derivative was prepared from 500 mg. of IV*c* and anhydrous methylamine in methanol by a procedure similar to that described for XII, except that IV*c* was added during 5 min.: yield of yellow precipitate, 267 mg. (44%); m.p. 105–115° dec.; IR bands at 1665s, 1610s, 1520mb, and 780mw. Analytical data were satisfactory for a hemihydrate of XIII but not for anhydrous material. It is likely that the material was not entirely pure, but further purification was not attempted because of the instability of XIII.

Anal.—Calcd. for $C_5H_8N_6O \cdot {}^1/_2H_2O$: C, 33.89; H, 5.12; N, 47.44. Found: C, 33.83; H, 5.05; N, 47.15.

A mixture of 150 mg. of XIII and 12 ml. of water–methanol (5:1) became homogeneous within 4.5 hr.; TLC after 5 hr. indicated that decomposition to III*c* was complete. Concentration of the solution *in vacuo* and trituration of the residual syrup with ether gave a yellow solid which was collected by filtration, washed with ether, and dried *in vacuo* at 56°: yield, 88 mg. (79%); m.p. 175–182°. Comparison of IR, UV, and TLC (5:3:2 butanol–H_2O–acetic acid) data from this material with those of an authentic sample of III*c* (m.p. 184–186°) showed the two samples to be identical.

3-[3,3-Bis(2-chloroethyl)-1-triazeno]pyrazole-4-carboxamide (XIV)—A solution of bis(2-chloroethyl)amine free base was prepared by the previously described (4) procedure, except that ethyl acetate was substituted for dichloromethane. The solution obtained from 8.85 g. of the hydrochloride and two 40-ml. portions of ethyl acetate was diluted with 8 ml. of anhydrous methanol; then 1.01 g. of IV*c* was added in portions, with stirring, during 30 min. The reaction mixture was stirred for 45 min., diluted with 50 ml. of cyclohexane, and filtered to remove a water-soluble precipitate (156 mg.). The filtrate was concentrated *in vacuo* to a volume of about 15 ml. and then diluted with 150 ml. of cyclohexane. A gummy solid precipitated; it was separated by decanting the supernatant liquid and was stirred for 15 min. with 50 ml. of a 1:1 mixture of ethyl acetate–cyclohexane. A granular solid was separated by filtration, washed with the same solvent mixture, and dried *in vacuo* at room temperature: yield, 1.30 g. (64%); m.p. 178–182° dec. An NMR analysis indicated that about 53% of this solid was the *v*-triazolinium salt formed from XIV. Therefore, the solid (1.2 g.) was stirred in 15 ml. of water at room temperature for 5 min., separated by filtration, washed with water, and dried *in vacuo* at 56° for 15 min. and at room temperature for 3.5 hr.: wt., 480 mg.; m.p. 110° with resolidification and remelting at 185–188° dec.; IR bands at 3375ms, 1645s, 1588ms, 1560sh, 1505mb, 780m, and 742mw. During TLC (9:1 $CHCl_3$–CH_3OH), some of the *v*-triazolinium salt was formed and appeared as a weak spot at the origin and as a streak to the fast moving major spot (XIV).

Anal.—Calcd. for $C_8H_{12}Cl_2N_6O$: C, 34.42; H, 4.33; N, 30.11; Cl, 25.40. Found: C, 34.58; H, 4.37; N, 29.93; Cl, 25.24.

An NMR analysis indicated that the quantity of the isomeric *v*-triazolinium salt present in this material was about 3–4% or less. This specimen was used in the D1 tests summarized in Table I. Although there was evidence of deterioration of some earlier specimens of XIV stored at low temperatures, the amount of the *v*-triazolinium salt in this specimen remained essentially unchanged during 4 months at −10°.

Stability Observations[5]—Observations of the relative stability of triazeno derivatives in solution were made by preparing the solutions in the dark in Pyrex volumetric flasks, withdrawing aliquot portions at convenient time intervals, diluting the aliquots with phosphate buffer (pH 7), and recording the UV spectra at pH 7. When the stabilities of the two compounds were to be compared, the solutions were placed side-by-side (with one exception) to equalize the conditions. The decrease in absorbance of the long wavelength maximum was considered to correspond to the disappearance of the triazeno derivative. The concomitant formation of a bicyclic *v*-triazinone (XVI, 2-azahypoxanthine, or 2,8-diazahypoxanthine) could be observed if substantial dissociation of an amide derivative occurred, but it was not established that the *v*-triazinone was the sole product. The difference between the wavelengths of the absorption maxima at pH 7 of 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (330 nm.) and 2-azahypoxanthine (286 nm., *Reference 1*) is large, and the absorbance of the latter compound is essentially zero at 330 nm. This difference is less for the pyrazole analog (X) (317 nm.) and the pyrazolo-*v*-triazinone (XVI) (doublet at 275 and 286 nm.), and absorption by XVI extends beyond the long wavelength maximum of X. There is more over-

[5] These experiments were performed to determine whether triazenopyrazoles are light sensitive and to compare compounds under like conditions. The data recorded here are presented in support of conclusions summarized in the *Discussion;* they should not be regarded as controlled rate determinations. In these experiments, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide decomposed in sunlight and in light in the 360–370-nm. region more rapidly than it had in earlier experiments (5) monitored by TLC, but exposure conditions differed. In the earlier experiments, the concentration was greater (25 mg./5 ml.), exposure to sunlight (in 50% methanol) occurred at different times of the year, and exposure of the 50% ethanol solution to light in the 365-nm. region was at a distance of 15 cm. (6 in.).

lapping of absorption in mixtures of X and XVI than in mixtures of 2-azahypoxanthine and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and less accuracy in estimating substantial decreases in the molar absorbance of X than of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide. There is essentially no absorption by 2,8-diazahypoxanthine ($UV_{max.}$ 278 nm.) at the long wavelength maximum (315 nm.) of the *v*-triazole analog (II). For all solutions placed in radiation in the 365-nm. region[6], the two solutions being compared were kept in a small dark room at room temperature (about 26–28°). Exposure to light caused increases of a few degrees in the temperature of the solutions[5]. The following experiments were performed according to the procedures and under the conditions described above.

Experiment 1—Solutions of X (about 4 mg.) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (about 6 mg.) in 50% aqueous ethanol contained in 25-ml. Pyrex volumetric flasks were placed side-by-side and exposed simultaneously to bright, direct sunlight passing through window glass. The temperature of the solutions reached 40° after 2 hr. The observed changes in absorbance (*A*) of X at 317 nm. were as follows (hr., percent change): 0.5, −7; 1, −9; 2, −31; and 3.5, −46. The disappearance of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide was virtually complete within 0.5 hr. (change of $A \geq 98\%$) and complete within 1 hr. The final spectra [after 1 hr. for 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and after further exposure of X the following day] indicated that XVI and 2-azahypoxanthine were the predominant products formed from X and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, respectively.

Experiment 2—Solutions of X (about 5 mg.) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (about 5 mg.) in 50% aqueous ethanol contained in 25-ml. Pyrex volumetric flasks were suspended side-by-side at a distance of 10 cm. (distance from the surface of the lamp filter to the wall of the flask) from a UV source[6] with maximum light emission at 365–366 nm. The changes in *A* of X at 317 nm. were as follows (hr., percent change): 0.5, −6; 1, −41; 2.5, −58; and 3.5, −71. The changes in 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide were the same as those described for Experiment 1.

Experiment 3—Solutions of X (14.6 mg.) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (9.9 mg.) in 50 ml. of a phosphate buffer of the Krebs–Ringer type were exposed to 365-nm. light as described in Experiment 2, except that the distance was 15 cm. The changes in *A* of X at 317 nm. were as follows (hr., percent change): 1, +3; 4, −5; 7, −8; 24, −31; 48, −62; 72, −88. The changes in *A* of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide at 330 nm. were as follows (hr., percent change): 1, −85; and 4, −99. The decreased rates of change in the Krebs–Ringer solution relative to those in 50% ethanol are probably due in part to the increased distance from the light source.

Experiment 4—Solutions of II (6.8 mg./50 ml.) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (12.5 mg./50 ml.) in 50% methanol were exposed to UV light as described in Experiment 2, except that the distance was 15 cm. and the two solutions were not irradiated simultaneously. The changes in *A* of II at 315 nm. were as follows (hr., percent change): 1, −25; 4, −93; and 6, −97. The disappearance of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide was almost complete within 1 hr. and complete within 2.5 hr. (A weak shoulder remained in the 330-nm. region after 1 hr., decrease in *A* about 96–97%.)

[6] The UV light source was a General Electric Bonus Line mercury reflector flood lamp, No. H100PFL 38-4, 100 watts (Hanovia Type 16106 power supply), fitted with a red-purple glass filter, Corning No. 5874, to remove most of the visible light. According to the manufacturer's data, the spectral radiant characteristics of the lamp and the transmission properties of the filter are such that about 80% of the radiation emitted by the lamp-filter combination should be between 360 and 370 nm. Since the mercury-emission spectrum has lines at 365–366 nm., the light emitted by the lamp-filter combination is assumed to be principally at 365–366 nm. No attempt was made to evaluate the influence of aging of the lamp and filter on the spectral properties of the emitted light.

Experiment 5—Solutions of VI (10 mg.) and methyl 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxylate (Ib, R = R_1 = R_2 = CH_3) (10 mg.) in 50 ml. of 50% aqueous ethanol were exposed to 365-nm. light as described in Experiment 2. The changes in *A* of VI at 313 nm. were as follows (hr., percent change): 1, −10; 3, −17; 7, −20; 25, −45; 50, −90; and 75, −100. The changes in *A* of the imidazole analog at 330 nm. were as follows (hr., percent change): 1, −8; 3, −28; 7, −64; 25, −99; and 50, −100. The final spectra showed maxima at 221 and 235 nm., respectively.

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