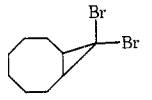
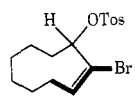
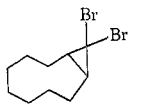
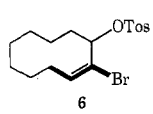
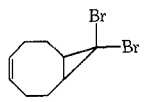
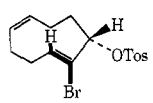
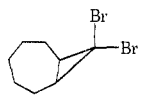
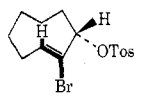
Table I

Starting compd	Product[a]	Mp, °C	Yield, %[b]	NMR data ($CDCl_3$), δ
 1	 5	91–93	93	6.04 (t, 1, olefin H, J = 8 Hz), 5.52 (dd, 1, methine H, J = 10 and 5 Hz), 2.40 (s, 3, CH_3)
 2	 6	108–111	81	5.75 (m, 2, olefin H and methine H), 2.41 (s, 3, CH_3)
 3	 7	89–91	89	5.85 (t, 1, olefin H, J = 8 Hz), 5.27 (m, 2, H cis double bond), 4.85 (dd, 1, methine H, J = 10 and 6 Hz), 2.39 (s, 3, CH_3)
 4	 8	80–82	85	6.10 (dd, 1, olefin H, J = 11 and 5 Hz), 4.92 (t, 1, methine H, J = 8 Hz), 2.42 (s, 3, CH_3)

[a] Satisfactory analytical data were obtained (±0.3% for C and H). [b] Yields are based upon isolated crude material (purity >95%).

triplets at δ 4.95 (J = 7 Hz) and 6.18 (J = 8.5 Hz). From these observations we must conclude that silver ion assisted ring opening of 1 and of 2 is leading initially to the free trans allylic cation which rapidly isomerizes to the cis allylic cation before reacting with the weakly nucleophilic tosylate anion.

The ring opening of 3 and of 4 leads exclusively to the trans allylic tosylates, because a free trans cation would require a sterically very unfavorable geometry, and therefore, these reactions must proceed completely concertedly leading to 7 and 8.

Experimental Section

General. Starting materials 1–4 were prepared by the reaction of dibromocarbene with the appropriate olefins according to known procedures.[9] *endo*-9-Bromobicyclo[6.1.0]nonane (11) was obtained from reduction of 1 with tri-*n*-butyltin hydride.[8] Silver tosylate was prepared from silver oxide and *p*-toluenesulfonic acid, according to the procedure of Kornblum et al.[10] NMR spectra were obtained on a Varian T-60 spectrometer using Me_4Si as an internal standard. The ring expansion reaction with silver tosylate is exemplified with the preparation of 7.

2-Bromo-3-tosyloxycyclonona-*trans,cis*-1,5-diene (7). To a solution of 2.80 g (0.01 mol) of 3 in 10 ml of acetonitrile was added a solution of 3.10 g (0.011 mol) of silver tosylate in 15 ml of acetonitrile. The mixture was stirred with gentle reflux for 2 hr. After cooling and addition of an equal volume of ether the precipitate was filtered and the filtrate evaporated to dryness. The resulting gummy product was chromatographed through a short silica gel column and afforded 3.3 g (89%) of white, crystalline tosylate 7. Recrystallization from diisopropyl ether gave analytical material, mp 89–91°. Anal. Calcd for $C_{16}H_{19}BrO_3S$: C, 51.75; H, 5.12. Found: C, 51.64; H, 5.12.

2-Bromo-3-hydroxycyclonon-1-ene (9, 10). To a solution of 5.64 g (0.02 mol) of 1 in 50 ml of 5% aqueous acetone was added a solution of 5.36 g (0.026 mol) of silver perchlorate in 25 ml of 5% aqueous acetone. The mixture was stirred at ambient temperature for 2 hr. After addition of 50 ml of saturated sodium chloride solution stirring was prolonged for an additional 5 min. The precipitate was filtered; the filtrate was diluted with 200 ml of water and extracted with ether. Upon washing, drying, and evaporation of the organic phase 3.19 g (73%) of the product was left as a colorless oil. The product consisted of two components. Chromatography (silica gel, chloroform–2% methanol as eluent) afforded 0.89 g of the cis alcohol **10** (R_f 0.29) and 2.29 g of the diastereomeric mixture of trans alcohols **9a,b**[2a] (R_f 0.22). The cis alcohol was recrystallized from petroleum ether: mp 72–74°; NMR ($CDCl_3$) δ 4.75 (m, 1, methine H), 6.18 (t, 1, olefin H, J = 11 Hz). Anal. Calcd for $C_9H_{15}BrO$: C, 49.31; H, 6.84. Found: C, 49.29; H, 6.80.

2-Bromo-3-hydroxycyclodec-1-ene (12, 13). A solution of 5.92 g (0.02 mol) of 2 and 5.36 g (0.026 mol) of silver perchlorate in 75 ml of 5% aqueous acetone was stirred for 3 hr at room temperature. Work-up in a similar manner as described for 9 and 10 afforded 3.21 g (71%) of product. Chromatography over silica gel (chloroform–2% methanol as eluent) afforded 2.55 g of trans alcohol 12 (R_f 0.38) as a colorless oil: NMR ($CDCl_3$) δ 4.18 (dd, 1, methine H, J = 9 and 5 Hz), 6.24 (t, 1, olefin H, J = 9 Hz). The other component (0.75 g, R_f 0.44) was the cis alcohol 13: mp 48–50° (petroleum ether); NMR ($CDCl_3$) δ 4.82 (t, 1, methine H, J = 8 Hz), 5.98 (dd, 1, olefin H, J = 12 and 6 Hz). Anal. Calcd for $C_{10}H_{17}BrO$: C, 51.50; H, 7.29. Found: C, 51.62; H, 7.31.

Registry No.—1, 32644-18-1; 2, 57129-79-0; 3, 54809-08-4; 4, 52750-35-3; 5, 57090-95-6; 6, 57090-96-7; 7, 57344-75-9; 8, 57344-76-0; 10, 32726-58-2; 12, 57090-97-8; 13, 57090-98-9; silver tosylate, 16836-95-6.

References and Notes

(1) C. B. Reese and A. Shaw, *J. Am. Chem. Soc.*, **92**, 2566 (1970).
(2) (a) C. B. Reese and A. Shaw, *Chem. Commun.*, 1365 (1970); (b) *ibid.*, 1367 (1970).
(3) D. Duffin and J. K. Sutherland, *Chem. Commun.*, 626 (1970).
(4) M. S. Baird and C. B. Reese, *Tetrahedron Lett.*, 4637 (1971).
(5) R. B. Woodward and R. Hoffmann, *J. Am. Chem. Soc.*, **87**, 395 (1965).
(6) C. H. de Puy, R. G. Snack, and J. W. Hamer, *J. Am. Chem. Soc.*, **88**, 3343 (1966).
(7) (a) P. v. R. Schleyer, W. F. Sliwinski, G. W. van Dine, U. Schollkopf, J. Paust, and K. Fellenberger, *J. Am. Chem. Soc.*, **94**, 125 (1972); (b) W. F. Sliwinski, T. M. Su, and P. v. R. Schleyer, *ibid.*, **94**, 133 (1972).
(8) M. S. Baird and C. B. Reese, *J. Chem. Soc. C*, 1808 (1969).
(9) L. Skattebøl, *Acta Chem. Scand.*, **17**, 1683 (1963).
(10) N. Kornblum, W. J. Jones, and G. J. Anderson, *J. Am. Chem. Soc.*, **81**, 4113 (1959).
(11) The two diastereoisomers of trans alcohol **12** equilibrate at room temperature at such a rate that only one NMR signal is observed.[2b]

Ring Opening of 5,7-Dimethyl-*v*-triazolo[1,5-*a*]pyrimidine by Halogenating Agents

Thomas Novinson,* Phoebe Dea, and Takayuki Okabe

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92664

Received June 13, 1975

In earlier publications, we demonstrated that electrophilic attack by halogens took place in the five-membered ring

of pyrazolo[1,5-*a*]pyrimidines[1,2] and pyrazolo[1,5-*a*]-1,3,5-triazines.[3] Although a few derivatives of *v*-triazolo[1,5-*a*]pyrimidine have been synthesized by Sutherland and Tennant,[4] little is known about the chemistry of this ring system. The site of electrophilic attack on similar 10 π electron (bridgehead nitrogen) heterocycles has been explored by Lynch et al.[5] and by Jacquier et al.,[6] and therefore such a study on 5,7-dimethyl-*v*-triazolo[1,5-*a*]pyrimidine (**1**) seemed appropriate from a theoretical viewpoint (as well as a biological viewpoint[2,7]).

When **1** was treated with *N*-bromosuccinimide (NBS) in chloroform, two products were isolated, neither of which was the expected 3-bromo-5,7-dimethyl-*v*-triazolo[1,5-*a*]pyrimidine (**9**). The major product, which was isolated in up to 90% yield, was assigned the structure of 2-(α,α-dibromomethyl)-4,6-dimethylpyrimidine (**2**), as determined by mass spectrum, elemental analysis, ^{1}H NMR, and ^{13}C NMR (see Experimental Section). The application of ^{13}C NMR and ^{1}H NMR in the structural determination was employed to rule out the possibility that **2** might be 5-bromo-2-(α-bromomethyl)-4,6-dimethylpyrimidine.

The minor product was assigned the structure of 4,6-dimethylpyrimidine-2-carboxaldehyde diethyl acetal (**3**), also consistent with elemental analysis, ir, uv, ^{1}H NMR, and MS data, as well as ^{13}C NMR spectra. A search of the literature indicated that pyrimidine-2-carboxaldehyde and its derivatives had not been reported. Data from the ^{13}C NMR spectrum were particularly useful for structure determination, in this respect, because the ring carbons of **2** and **3** had essentially identical shieldings, but the signal for the α carbon of **3** occurred 51 ppm downfield from the α carbon of **2** (e.g., the substituted 2-methyl group of **2** and **3**). Such large deshielding effects had been demonstrated by Lauterbur[8] in alkoxy oxygen atoms. The acetal carbon in the ^{13}C NMR spectrum of neat methylaminoacetaldehyde dimethyl acetal was found to have the same shift (102.9 ppm) as that of **3**.

The origin of **3** presented a problem. Refluxing **2** in ethanol did not yield **3**, nor did **3** arise from adding NBS and a trace of ethanol to a solution of **2** in chloroform. It appeared that **3** did originate from a trace amount of ethanol in the solvent (when freshly dried chloroform was used, **3** was not isolated), but the addition of more ethanol during the bromination reaction did not substantially increase the yield of **3**. The bromination of **1** in methylene chloride with bromine at 0°, and in sodium hydroxide with bromine at 0°, gave only **2**.

In contrast to the above reactions, the bromination of **1** in ethanol at −20° gave a mixture of five products. From the GLC data of the crude mixture, it appeared that the decomposition of **1** was quantitative, giving approximately 60% of **3**, 10% of **2**, 20% of a derivative of the *v*-triazolo[1,5-*a*]pyrimidine ring, 9% of one new pyrimidine derivative, and 1% of another new pyrimidine.

Elemental analysis, uv, ir, and ^{1}H NMR substantiated the structure of one of the isolated products as being 3-bromo-5,7-dimethyl-*v*-triazolo[1,5-*a*]pyrimidine (**9**). As reported elsewhere,[6,7] the unique fluorescence of certain bridgehead nitrogen heterocycles (long-wavelength uv; silica gel TLC plate, as described in the Experimental Section), along with supporting uv data (e.g., the comparison of the uv data of **1** and **9**), helped to establish that the original ring system was still intact in compound **9**.

The remaining two products were identified by elemental analyses and spectra (ir, uv, and ^{1}H NMR). The material obtained in 9% yield was assigned the structure of 4,6-dimethyl-2-ethoxymethylpyrimidine (**8**) and the compound obtained in 1% yield was identified as 2-(α-bromoethoxymethyl)-4,6-dimethylpyrimidine (**7**). The latter (**7**) was not very stable and was also found to convert to **3** upon standing in ethanol at 25°, perhaps suggesting that **3** originated from **7** in the original reaction.

The reaction of **1** with *N*-chlorosuccinimide (NCS) gave the expected 2-(α,α-dichloromethyl)-4,6-dimethylpyrimidine (**5**). Normally, the use of iodine monochloride in the halogenation of nitrogen bridgehead heterocycles gives substitution of the ring with iodine, and hydrogen chloride is released.[2] When **1** was treated with this reagent, the product obtained was 4,6-dimethyl-2-(α-iodo-α-chloromethyl)pyrimidine (**4**).

It is possible that **1** may be in equilibrium with, or at least may coexist with, a 4,6-dimethylpyrimidinyl-2-diazomethane (**6**). Although we were unable to detect this species via low-temperature ^{1}H NMR and ^{13}C NMR spectra, such a structure is plausible. Sutherland and Tennant[4] suggested that a "diazonium cation" accounted for the decomposition of the 3-phenyl derivative of **1** in acetic acid. A similar equilibrium was demonstrated by Temple et al.[9,10] for 5,7-dimethyltetrazolo[1,5-*a*]pyrimidine, which was found to coexist with the 2-azidopyrimidine. Additionally, our earlier work[11] on the equilibria of the 8-phenyltetrazolo[1,5-*e*]pyrazolo[1,5-*a*]-1,3,5-triazine ring in the presence of bromine also supports this hypothesis.

All of the products appear to be consistent with a cationic mechanism, involving standard diazoalkane electrophile reactions,[12] as illustrated in Scheme I. Solvolysis by ethanol apparently accounts for **3** and **8**. From a general synthetic viewpoint, the reaction of diazoalkyl compounds with halogens to yield α,α-dihalomethyl derivatives has been of limited value in previous applications. However, the high yields of **2**, **4**, and **5** indicate that the present method is a potentially useful synthetic route to 2-substituted pyrimidines that are inaccessible or difficult to prepare by other methods.

Experimental Section

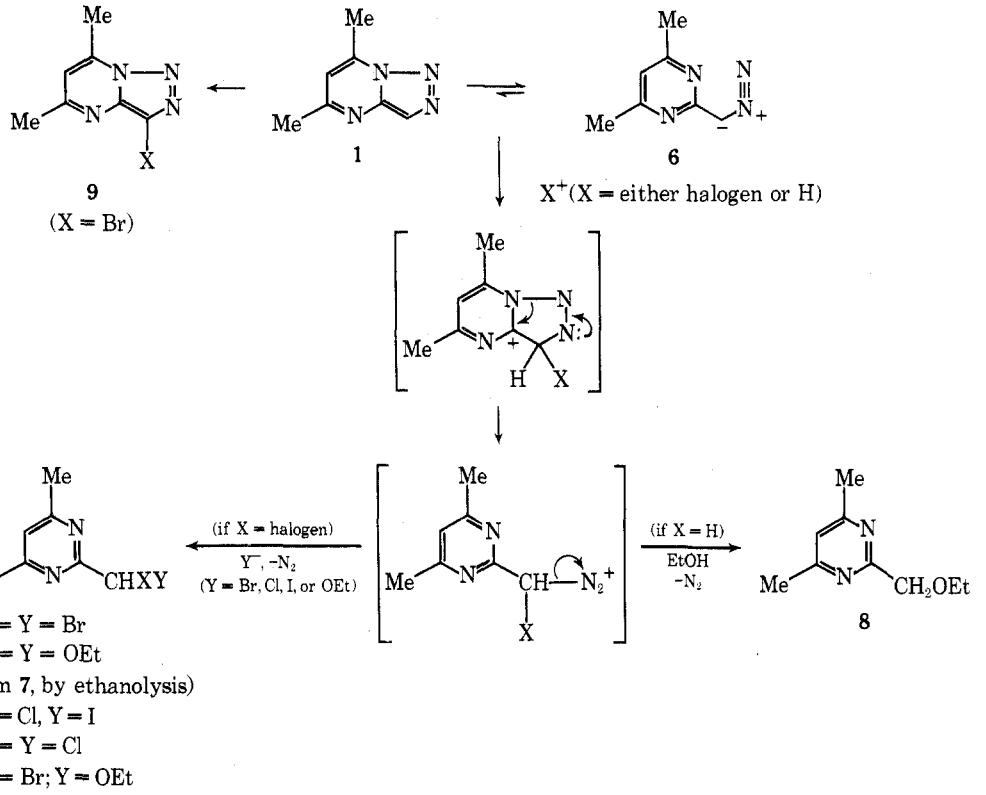
^{13}C NMR spectra were taken in 20% $CDCl_3$ solutions and recorded on a Bruker HX-90 spectrometer operating at 22.62 MHz in the Fourier transform mode at a probe temperature of 35°. A Nicolet 1074 signal averager with 4096 word memory was used for data accumulation and a PDP-8/e computer (Digital Equipment Corp.) for data processing. Chemical shifts were measured from $CDCl_3$ and then converted to the tetramethylsilane (Me_4Si) scale using the relationship $\delta_{Me_4Si} = \delta_{CDCl_3} + 77.2$ ppm. ^{1}H NMR spectra were taken in $CDCl_3$ with Me_4Si as an internal standard, recorded on a 60-MHz Hitachi Perkin-Elmer R20A spectrometer. Ir spectra were obtained in KBr disks if the sample was solid, and as a thin layer on NaCl cells if liquid, both being obtained on a Perkin-Elmer 257 spectrophotometer. Mass spectra were obtained on a Perkin-Elmer 270 high-resolution spectrometer. Gas–liquid chromatography was obtained on a Varian Model 2100 gas chromatograph equipped with flame ionization detector. A 3.8 mm × 6 ft glass column packed with 3% OV-101 was used at a column temperature of 220° and detector at 250°. Column chromatographic separations were performed on Woelm activity grade I neutral alumina, eluted with chloroform, unless otherwise noted. Analyses and molecular weight determination were performed by Galbraith Laboratories, Knoxville, Tenn.

5,7-Dimethyl-*v*-triazolo[1,5-*a*]pyrimidine (1). A mixture of 0.84 g (0.01 mol) of 5-amino-*v*-triazole[13] and 1.0 g (0.01 mol) of acetylacetone was refluxed in 10 ml of ethanol containing 2 drops of piperidine, for a period of 1 hr. The resulting solution was allowed to cool to room temperature and then it was evaporated at 25° (0.1 mm) to yield a gummy residue. The crude product was recrystallized from benzene–petroleum ether to yield 1.1 g (75%) of the product in the form of yellowish-white needles, mp 82–84°. The condensation was also performed in the absence of solvent (no piperidine), giving a 70% yield, and also in benzene, giving an 81% yield: mp 83–84°; ^{1}H NMR ($CDCl_3$) 2.67 (s, 3), 2.92 (s, 3), 6.78 (s, 1), and 8.16 (s, 1).

Anal. Calcd for $C_7H_8N_4$ (148.16): C, 56.74; H, 5.44; N, 37.82. Found: C, 57.02; H, 5.60; N, 38.05.

Bromination of 1. 2-(α,α-Dibromomethyl)-4,6-dimethylpy-

Scheme I



2, X = Y = Br
3, X = Y = OEt
(from 7, by ethanolysis)
4, X = Cl, Y = I
5, X = Y = Cl
7, X = Br; Y = OEt

rimidine (2). Method A. A solution of 1.0 g 1 in 50 ml of methylene chloride was cooled to 0° and 1.1 g of bromine was added dropwise over a 10-min period. The solvent was evaporated and the residue was recrystallized from methanol–water to yield 0.9 g (90%) of 2-dibromomethyl-4,6-dimethylpyrimidine (2) as yellowish-white needles, mp 82–84°. A second recrystallization, from ether, gave white cubettes, melting point unchanged. A mixture melting point depression of 60–65° was recorded when 1 and 2 were mixed.

Uv (MeOH) λ_{max} 225 nm (ϵ_{max} 6780), 250 sh (6510); ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1), 6.64 (s, 1), and 2.53 (s, 6); ^{13}C NMR ($CDCl_3$) 167.7 (C_2), 165.6 (C_4, C_6), 119.8 (C_5), 23.9 (Me at C_4, C_6) and 41.9 ppm ($CHBr_2$ at C_2).

Anal. Calcd for $C_7H_8N_2Br_2$ (279.96): C, 30.03; H, 2.88; Br, 57.08. Found: C, 29.82; H, 2.83; Br, 57.20.

Method B. A solution of 0.9 g of 1 in 50 ml of chloroform was cooled to 0° and 1.1 g (0.01 mol) of *N*-bromosuccinimide was added in small portions. The solution was then washed with ice-cold 1*N* NaOH (~ 100 ml), followed by water, and dried over Na_2SO_4. The chloroform was then evaporated at 20° (8 mm) to yield an oily residue. Chromatography of this material on alumina (Woelm, neutral, activity grade I) with chloroform gave two fractions. The first fracton, upon evaporation of the solvent, gave 120 mg (20%) of 2 as white needles, mp 82–84°. The second fraction, upon evaporation, gave 200 mg of a colorless oil which was judged sufficiently pure for analysis (GLC, as stated earlier). This material was shown to be **4,6-dimethylpyrimidine-2-carboxaldehyde diethyl acetal (3):** bp 78–80 (0.1 mm); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6), 2.5 (s, 6), 3.75 (q, 4), 5.5 (s, 1), and 7.0 (s, 1); ^{13}C NMR ($CDCl_3$) 167.1 (C_2), 165.2 (C_4, C_6), 119.6 (C_5), 23.9 (Me at C_4, C_6), 102.9 [$CH(OEt)_2$], 62.8 (OCH_2CH_3), 15.2 ppm (OCH_2CH_3); uv (MeOH) λ_{max} 250 nm (ϵ_{max} 8530).

Anal. Calcd for $C_{11}H_{18}N_2O_2$ (198): C, 60.58; H, 9.15; N, 14.13. Found: C, 60.40; H, 8.80; N, 14.44.

Method C. *A solution of 1.0 g of 1 in 50 ml of water containing* 1.0 g of sodium hydroxide was cooled to 0°. Then 1 ml of bromine was added dropwise over a 5-min period. The mixture was stirred for 30 min at 0°, then extracted with methylene chloride. The solvent was dried ($MgSO_4$) and evaporated. The residual oil was chromatographed on neutral alumina, as in method B. Only one fraction was obtained, and this gave 350 mg (40%) of 2, mp 82–84°, upon work-up (following the procedure given above).

4,6-Dimethyl-2-(α-iodo-α-chloromethyl)pyrimidine (4). A solution of 1.0 g of 1 in 50 ml of chloroform was cooled to 0° and 1.1 g of iodine monochloride was added, in small portions. Vigorous ebullition of nitrogen gas was observed at this temperature. The dark mixture was refrigerated for 24 hr, then filtered through 50 g of neutral alumina and eluted with chloroform. Upon evaporation of the eluent, a yellow oily residue was obtained, which soon solidified. Recrystallization of this solid from petroleum ether gave 1.2 g (48.6%) of pale yellow cubettes: mp 73–75°; ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 6), 6.83 (s, 1), and 6.94 (s, 1); uv (MeOH) λ_{max} 230 nm (ϵ_{max} 7200), 260 sh (6830).

Anal. Calcd for $C_7H_8N_2ClI$ (282.5): C, 29.76; H, 2.85; N, 9.92. Found: C, 29.72; H, 2.88; N, 9.60.

2-(α,α-Dichloromethyl)-4,6-dimethylpyrimidine (5). A solution of 1.5 g (0.01 mol) of 1 in 70 ml of chloroform was cooled to −20° (dry ice–acetone bath) and 1.4 g (0.011 mol) of *N*-chlorosuccinimide in 20 ml of chloroform was added dropwise over a 5-min period. The mixture was stirred for 2.5 hr at −20°, then stored at 0° for 20 hr. The solution was then washed with aqueous sodium bicarbonate and the organic extract dried ($MgSO_4$) and evaporated in vacuo to yield an oily residue. Chromatography of the material on neutral alumina with chloroform gave 100 mg (10%) of the product, recrystallized from petroleum ether as white needles: mp 77–78°; ^{1}H NMR ($CDCl_3$) 2.55 (s, 6), 6.70 (s, 1), and 7.03 (s, 1); uv (MeOH) λ_{max} 228 nm (ϵ_{max} 7100), 250 sh (6200).

Anal. Calcd for $C_7H_8N_2Cl_2$ (191.1): C, 44.01; H, 4.22; N, 14.66. Found: C, 44.20; H, 4.35; N, 14.70.

Bromination of 1 in Ethanol. A solution of 1.0 g (6.7 mmol) of 1 in 25 ml of EtOH was cooled to −20° and 1.1 g of bromine in 10 ml of EtOH was added dropwise over a 10-min period, with stirring. A yellow precipitate formed, which decomposed with vigorous evolution of nitrogen, upon reaching 15–20°. The resultant clear, yellow solution was concentrated in vacuo to yield a gummy residue. The gum was dissolved in CH_2Cl_2 (50 ml) and washed with aqueous $NaHCO_3$ and then water, and the organic phase was dried ($MgSO_4$). Evaporation of the solvent gave an oil which was analyzed both by GLC and by TLC (percent isolated and R_f values are given for each compound below).

The crude mixture was chromatographed, giving in order of elution ($CHCl_3$) **2, 9,** and **3.**

(a) **2** (130 mg, 8%, GLC 10%, R_f 0.79), mp 75–77° (ir and ^{1}H NMR compared with original).

(b) **3-Bromo-5,7-dimethyl-*v*-triazolo[1,5-*a*]pyrimidine (9)** (100 mg, 10%, GLC 20%, R_f 0.56), mp 103–104° dec, recrystallized from petroleum ether (bp 30–60°).

Anal. Calcd for $C_7H_7N_4Br$: C, 37.02; H, 3.10; N, 24.67. Found: C, 37.08; H, 3.16; N, 24.33.

^{1}H NMR ($CDCl_3$) δ 2.79 (s, 3), 2.90 (s, 3), 6.80 (s, 1); uv (MeOH)

λ_{max} 223 nm (ϵ_{max} 27120), 274 (3890), 284 (39740), and 310 (3200); ir (KBr) 1630, 1450, 1383, 1525, and 1322 cm^{-1}.

(c) **3** (400 mg, 43%, GLC 70%, R_f 0.43), bp 93–95 (0.5 mm) and 78–80 (0.1 mm), which was identical with the material obtained from the chloroform reaction (^1H NMR, uv, and ir).

Acknowledgment. The authors are grateful to L. Larek for mass spectra and GLC data, and to E. Banta and M. Strikaitis for other spectra. We would also like to thank R. Day for technical assistance and Drs. R. K. Robins, R. B. Meyer, Jr., L. N. Simon, B. Bhooshan, and A. A. Albert for helpful suggestions.

Registry No.—**1**, 57173-97-4; **2**, 57173-98-5; **3**, 57173-99-6; **4**, 57174-00-2; **5**, 57174-01-3; **7**, 57174-02-4; **9**, 57174-03-5; 5-amino-*v*-triazole, 30132-90-2; acetylacetone, 123-54-6; *N*-bromosuccinimide, 128-08-5; iodine monochloride, 7790-99-0; *N*-chlorosuccinimide, 128-09-6.

References and Notes

(1) T. Novinson, R. K. Robins, and D. E. O'Brien, *Tetrahedron Lett.*, 3149 (1973).
(2) T. Novinson, R. Hansen, M. K. Dimmitt, L. N. Simon, R. K. Robins, and D. E. O'Brien, *J. Med. Chem.*, **17**, 645 (1974).
(3) T. Novinson, K. Senga, J. Kobe, R. K. Robins, D. E. O'Brien, and A. A. Albert, *J. Heterocycl. Chem.*, **11**, 691 (1974).
(4) D. R. Sutherland and G. Tennant, *Chem. Commun.*, 1070 (1969), and references cited therein.
(5) B. Lynch, M. A. Khan, S. C. Sharma, and H. C. Leo, *Can. J. Chem.*, **53**, 119 (1975), and earlier references cited therein.
(6) R. Jacquier, H. Lopez, and G. Maury, *J. Heterocycl. Chem.*, **10**, 755 (1973).
(7) T. Novinson, J. P. Miller, M. Scholten, R. K. Robins, L. N. Simon, D. E. O'Brien, and R. B. Meyer, Jr., *J. Med. Chem.*, **18**, 460 (1975).
(8) P. C. Lauterbur, *Ann. N.Y. Acad. Sci.*, **70**, 841 (1958).
(9) C. Temple, C. L. Kussner, and J. A. Montgomery, *J. Org. Chem.*, **31**, 2210 (1966), and references cited therein.
(10) C. Temple, W. C. Coburn, M. C. Thorpe, and J. A. Montgomery, *J. Org. Chem.*, **30**, 2395 (1965).
(11) J. Kobe, D. E. O'Brien, R. K. Robins, and T. Novinson, *J. Heterocycl. Chem.*, **11**, 991 (1974).
(12) W. J. Hickinbottom, Ed., "Reactions of Organic Compounds", 3rd ed, Wiley, New York, N.Y., 1962, pp 510–512, and references cited therein.
(13) P. N. Neuman, *J. Heterocycl. Chem.*, **7**, 1159 (1970).

Fluorometric Reagents for Primary Amines. Syntheses of 2-Alkoxy- and 2-Acyloxy-3(2*H*)-furanones

Manfred Weigele,* John P. Tengi, Silvano De Bernardo, Ronald Czajkowski,[1] and Willy Leimgruber

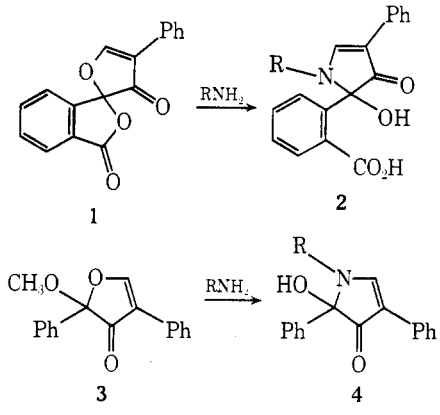
Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Received September 3, 1975

In recent years, fluorescamine, 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (**1**),[2] has become a widely used reagent for the fluorometric quantitation of primary amines. Fluorescamine reacts with primary amines (RNH_2) to form pyrrolinones of type **2** which upon excitation at 390 nm emit strong fluorescence at 475–490 nm (Scheme I). This reaction proceeds efficiently at room temperature in aqueous solutions[3] and allows the fluorometric estimation of submicromolar concentrations of amines, notably those of biological importance.[4] Many specific analytical applications of fluorescamine have been described in the recent literature, among them highly sensitive procedures for automated amino acid analyses[5,6] and for the assay of proteins.[7] Most recently, the use of fluorescamine has also been suggested for the colorimetric assay of amino acids.[8]

The structurally related compound, 2-methoxy-2,4-diphenyl-3(2*H*)-furanone (MDPF, **3**), which reacts similarly

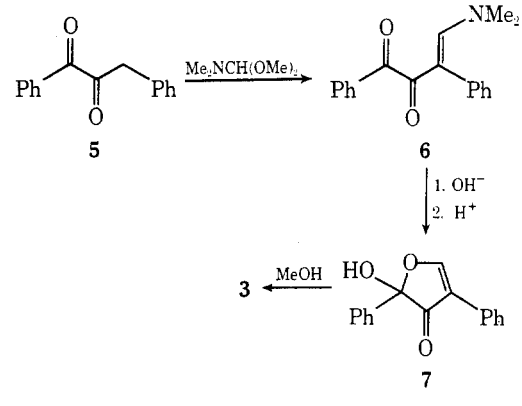
Scheme I



with primary amines to give fluorescent products of type **4**, was found particularly suitable for the fluorescent labeling of proteins and has been used in the preparation of fluorophoric immunoglobulin conjugates.[9] MDPF (**3**) has also been employed to derivatize α-amino acids for the purpose of determining their absolute configuration by chiroptical means.[10]

In this report we wish to describe in detail the syntheses of fluorescamine (**1**) and MDPF (**3**). As depicted in Schemes II and III, the carbon skeleton of these compounds was readily constructed by formylation of suitably substituted 1,2-propanediones.

Scheme II



Thus, in the case of MDPF (**3**), 1,3-diphenyl-1,2-propanedione (**5**)[11] was converted by reaction with *N,N*-dimethylformamide dimethyl acetal to the dimethylaminomethylene derivative **6** (Scheme II). Alkaline hydrolysis of this enamine, followed by acidic work-up, gave the hydroxyfuranone **7**, which was smoothly converted to the desired methoxy derivative **3** by heating in methanol at reflux temperature. Proof for the structure of **3**, in particular the establishment of its cyclic nature, has already been outlined in a previous communication.[2]

The hydroxycinnamoylbenzoic acid **11**, which was required for the synthesis of **1**, was initially obtained by hydrolysis of 3-benzylidene-1,4-isochromandione (**10**)[2] (Scheme III). However, the preparation of **10** according to literature procedures[12] was found to be cumbersome and inefficient. We therefore chose to prepare **11** by a novel route. Thus, 2-benzylideneindan-1,3-dione (**8**)[13] was converted by base-catalyzed oxidation with hydrogen peroxide in methanol to the epoxide **9**. Hydrolysis of **9** with sodium hydroxide led to cleavage of both the indandione and the oxirane ring (cf. **9**, arrows) to afford the desired 1,2-propanedione derivative **11**. The formylation of **11** was carried