

Figure 1. Thymidylate kinase time-course study (for conditions, see text).

5'-(4-Carboxy-1,2,3-triazol-1-yl)-5'-deoxythymidine (17). 16 (600 mg) was dissolved in 1 M sodium hydroxide (10 ml) and left at room temperature for 16 hr. The mixture was acidified with a few drops of 10 N hydrochloric acid. The crystals obtained on standing were collected by filtration to give 17: 0.45 g (81%); mp 222–224° dec; uv max 265 nm (ϵ 8400); nmr δ 6.14 (t, 1'-H), 8.55 (s, 5-H). *Anal.* (C₁₃H₁₅N₅O₆) C, H, N.

5'-(4-Carbamoyl-1,2,3-triazol-1-yl)-5'-deoxythymidine (18). 16 (4 g, 1.2 mmol) was dissolved in dry methanol (200 ml) and saturated with ammonia at 0°, and the mixture was left at room temperature for 48 hr. The solid that deposited was collected by filtration to give 18: 2.8 g (76%); mp 243–245° dec; uv max 265 nm (ϵ 8500); nmr δ 6.16 (t, 1'-H), 8.46 (s, 5-H). *Anal.* (C₁₃H₁₆N₆O₅) C, H, N.

3'-O-Acetyl-5'-(4-carbamoyl-1,2,3-triazol-1-yl)-5'-deoxythymidine (19). 18 (2.4 g, 1.2 mmol) was dissolved in dry pyridine (400 ml) and acetic anhydride (2.4 ml, 0.025 mmol) added. The mixture was left at room temperature for 48 hr and the pyridine removed by evaporation *in vacuo*. The residue was dissolved in chloroform and the mixture shaken with water. The chloroform layer was dried over magnesium sulfate and filtered, and the filtrate was evaporated to a solid which crystallized from ethanol to give 19: 2.1 g (77%); mp 259–260°; nmr δ 6.14 (t, 1'-H), 8.59 (s, 5-H). *Anal.* (C₁₅H₁₈N₆O₆) C, H, N.

5'-(4-Cyano-1,2,3-triazol-1-yl)-5'-deoxythymidine (21). 19 (1.9 g, 0.57 mmol) was dissolved in dry pyridine (200 ml) and phosphoryl chloride (1.4 ml, 15.7 mmol) added. The red solution was stirred at –5° for 3 hr and the solvent evaporated *in vacuo* at 10°. The oily residue was dissolved in acetonitrile and fractionated on a column of silicic acid (20 × 2 cm) with a mixture of acetonitrile–water (49:1 v/v) as eluent. Fractions containing the major product were pooled and evaporated to give 20 as a white solid (1.6 g). This was treated without further purification with a solution of 17.5% aqueous ammonia (v/v, 50 ml) at room temperature for 1 hr. The solvent was evaporated *in vacuo* and the solid thus obtained was crystallized from aqueous ethanol to give 21: 0.89 g (54%); mp 262° dec; uv max 264 nm (ϵ 8900); nmr δ 6.12 (t, 1'-H), 9.00 (s, 5-H). *Anal.* (C₁₃H₁₄N₆O₄) C, H, N.

Preparation of the Enzyme and Assay Method. Thymidylate kinase was prepared following the method of Langen and Kowollik.¹ The enzyme was isolated from Ehrlich ascites carcinoma cells‡ collected 6–10 days after passage of the tumour. The cells were sonified in 4 vol of 5.3 mM Tris–Cl buffer (pH 7.4) containing 0.66 mM MgCl₂ and 1 mM mercaptoethanol for 30 sec. The homogenate was spun at 178,000g for 40 min and the supernatant used as a crude source of thymidylate kinase.

The enzyme was assayed according to the method of Behki and Schneider.⁶ The assay mixture was as follows: 10 mM ATP, 10 mM MgCl₂, 15.8 mM sodium phosphoglycerate, 80 mM Tris–Cl buffer (pH 7.4), 0.12 mM substrate (50,000 cpm of [3H]thymidine monophosphate), and 50 μ l of enzyme diluted (1:1) with buffer in

a total volume of 100 μ l. Reactions were stopped with 10 μ l of cold 32% trichloroacetic acid. The samples were washed with 3 × 1 ml of water-saturated ether and spun for 2 min in a microcentrifuge (Eppendorf). Aliquots of the samples (20 μ l) were applied to PEI cellulose plates and separated using 2 M formic acid–0.5 M LiCl (1:1) as the solvent system.⁷ Spots identified under uv as corresponding to thymine, TMP, TDP, and TTP were cut out and counted in 8.5 ml of toluene–butyl PBD (0.8%). A time-course study was carried out for thymidylate kinase and the results are given in Figure 1. Approximately 50% conversion of substrate was found after 15 min for thymidylate kinase (at 37°); so this incubation time was used to study the effects of the analogs.

Acknowledgments. The authors wish to thank Dr. G. Kowollik, Akademie der Wissenschaften der DDR, GDR-1115, Berlin-Buch, for his generous gift of 5'-fluoro-5'-deoxythymidine. Thanks are also due to Mr. D. Greatbanks of this Division for his interpretation of the nmr spectra and to Dr. R. A. Bucknall for the biological test results.

References

- (1) P. Langen and G. Kowollik, *Eur. J. Biochem.*, **6**, 344 (1968).
- (2) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149 (1963).
- (3) J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).
- (4) J. P. Neenan and W. Rohde, *J. Med. Chem.*, **16**, 580 (1973).
- (5) R. A. Bucknall, H. Moores, R. Simms, and B. Hesp, *Antimicrob. Ag. Chemother.*, **4**, 294 (1973).
- (6) R. M. Behki and W. C. Schneider, *Biochem. Biophys. Acta.* **68**, 34 (1966).
- (7) K. Randerath and E. Randerath, *J. Chromatogr.*, **16**, 111 (1964).

3-Onium Derivatives of 1,4-Benzodiazepin-2-ones with Tertiary Organic Bases

T. Kovač, F. Kajfež,* V. Šunjić, and M. Oklobdžija

Compagnia di Ricerca Chimica S. A., Chiasso, Switzerland, and Institute of Organic Chemistry and Biochemistry, University of Zagreb, 41000 Zagreb, Croatia, Yugoslavia.

Received December 19, 1973

Chemistry. 1,4-Benzodiazepin-2-ones can be formed under mild conditions by cyclization of some acyclic, more hydrophilic precursors of the resulting pharmacons.^{1,2} Using such hydrophilic precursors of 1,4-benzodi-

‡Kindly provided by Dr. B. Fox, Christie Hospital, Manchester.

Table I. 3-Onium Derivatives of 7-Chloro-5-substituted Phenyl-1,4-benzodiazepin-2-ones^a

No.	R ₁	R ₂	R ₃	Recrystn solvent	Yield, %	Mp, °C ^b	Formula ^c
VII	H	Cl		Acetone	90	234–237	C ₂₀ H ₁₄ Cl ₃ N ₃ O
VIII	H	H		Water	80	234–236	C ₂₀ H ₁₅ Cl ₂ N ₃ O
IX	CH ₃	H		Acetone	80	229–231	C ₂₁ H ₁₇ Cl ₂ N ₃ O
X	H	Cl		Acetone	78	260–263	C ₁₉ H ₁₅ Cl ₃ N ₄ O
XI	H	H		Acetone	70	215–218	C ₁₉ H ₁₆ Cl ₂ N ₄ O
XII	CH ₃	H		Acetone	80	209–212	C ₂₀ H ₁₈ Cl ₂ N ₄ O
XIII	H	Cl		Acetone	93	256–260	C ₂₀ H ₁₇ Cl ₃ N ₄ O
XIV	H	H		Acetone	67	268–272	C ₂₀ H ₁₈ Cl ₂ N ₄ O
XV	CH ₃	H		Acetone	60	230–233	C ₂₁ H ₂₀ Cl ₂ N ₄ O
XVI	H	Cl		Acetone	80	261–263	C ₂₁ H ₁₅ Cl ₃ N ₄ O ₂
XVII	H	Cl		Acetone	90	222–224	C ₂₁ H ₁₅ Cl ₃ N ₄ O ₂
XVIII	CH ₃	H		Acetone	80	273–276	C ₂₂ H ₁₈ Cl ₂ N ₄ O ₂
XIX	H	Cl		Acetone	86	182–185	C ₂₅ H ₂₃ Cl ₃ N ₄ O ₂
XX	H	H		Acetone	70	200–203	C ₂₅ H ₂₄ Cl ₂ N ₄ O ₂
XXI	CH ₃	H		Acetone	70	169–172	C ₂₆ H ₂₆ Cl ₂ N ₄ O ₂
XXII	H	Cl		Acetone	70	168–170	C ₂₁ H ₂₂ Cl ₃ N ₃ O ₃
XXIII	H	H		Acetone	50	169–172	C ₂₁ H ₂₃ Cl ₂ N ₃ O ₃
XXIV	H	Cl		Acetone	70	234–237	C ₂₁ H ₁₇ BrCl ₃ N ₅ O ₃
XXV	H	H		Acetone– methanol	70	209–211	C ₂₁ H ₁₈ BrCl ₂ N ₅ O ₃
XXVI	CH ₃	H		Acetone– methanol	60	199–202	C ₂₂ H ₂₀ BrCl ₂ N ₅ O ₃
XXVII	H	Cl		Acetone	70	216–219	C ₂₂ H ₁₆ Cl ₃ N ₃ O ₃
XXVIII	H	H		Acetone	67	237–239	C ₂₀ H ₂₁ Cl ₂ N ₃ O ₂
XXIX	H	Cl		Ethyl acetate	70	168–170	C ₃₅ H ₃₂ Cl ₃ N ₃ O
XXX	H	Cl		Acetone	85	198–201	C ₂₇ H ₂₅ Cl ₄ N ₃ O ₄

^aAll compounds listed below exhibited in the nmr spectrum a characteristic singlet for the 3-C(H)N⁺ proton at 6.36–6.90 ppm, which exhibited H/D exchange when dissolved in D₂O or CD₃OD. Characteristic bands in infrared spectra were regularly found at 1695, 1620, 1595, 1565, 1498, 1450, 1330, 748, and 700 cm⁻¹. ^bAll compounds melted under decomposition above the temperatures indicated. ^cElemental analyses for N were performed. The results were within ±0.4% of the theoretical values. ^dPrepared according to F. Kajfež, *et al.*, *J. Med. Chem.*, **11**, 167 (1968). ^eFree base has been used therapeutically (antidepressant; generic name amitriptyline). ^fFree base has been used therapeutically (analeptic; generic name centrophenoxine).

azepines some useful alternations of their absorption *in vivo* can be achieved. Here we report a preparation of a new set of hydrophilic 3-substituted 1,4-benzodiazepin-2-one derivatives VII–XXX which could be hydrolyzed under the physiological conditions into 3-hydroxy derivatives I–III with known pharmacological properties.^{3,4} These compounds comprise tertiary organic bases quaternized with 3-chloro-1,4-benzodiazepin-2-ones IV–VI. Some physical and pharmacological data of the compounds VII–XXX are listed in Tables I and II. Detailed investigations

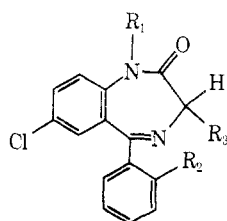
in the pH range 1–12 revealed unexpected stability of the C(3)–NR₃⁺ bond to solvolysis; formation of minor quantities of 3-hydroxy compounds I–III along with strong decomposition was observed above pH 11 at room temperature [tlc monitoring, chloroform–acetone (9:1) as eluent]. Preliminary *in vitro* investigations, however, as performed using rat liver homogenate (9000g supernatant) as a biocatalytic medium (pH 7.2, 35 ± 1°) for conversion of the compounds VII, XI, XV, XVI, and XVIII revealed formation of 3-hydroxy compounds in all cases under investiga-

Table II. Pharmacological Potencies of 3-Onium Derivatives of 1,4-Benzodiazepin-2-ones and Selected Reference Agents (Relative Potencies in Comparison with Chlordiazepoxide)

No.	Anticonvulsant effect			Muscle relaxation	Fighting test	Hypnotic effect	LD ₅₀ , po, mg/kg
	Pentylene tetrazole	Electroshock					
		Max	Min				
D ^a	5.8	6.2	1.73	3.30	4.3	1.75	0.800
M ^a	6.2	0.81	0.32	1.3	1.0	0.30	1.420
III ^a	12.3	2.1	0.77	0.44	1.3	0.48	4.000
VII	13.6	3.5	1.0	1.0	1.0	0.3	1.750
VIII	17.0	4.0	1.1	1.0	2.5	2.3	1.520
IX	15.6	3.7	0.9	0.9	2.3	2.3	1.600
X	5.3	5.7	1.6	3.5	4.2	2.0	1.250
XI	5.6	6.0	2.0	3.9	4.5	2.1	1.380
XII	7.5	7.9	2.3	4.0	4.6	3.2	1.100
XIII	4.3	4.7	0.90	3.0	3.9	1.7	1.420
XIV	5.9	6.3	2.5	4.0	4.2	3.0	1.130
XV	5.0	5.6	2.0	0.5	0.6	1.7	0.400
XVI	6.0	0.70	0.31	1.0	0.6	0.40	1.680
XVII	5.8	0.60	0.20	0.3	0.6	0.30	1.960
XVIII	6.5	0.80	0.42	1.2	0.7	0.40	1.970
XIX	7.3	1.6	1.30	1.5	1.3	1.6	2.300
XX	7.0	1.2	1.0	1.3	1.0	1.5	2.480
XXII	20.5	10.3	3.7	6.30	7.2	2.6	0.850
XXIII	19.0	9.6	3.2	5.9	6.8	2.0	0.980
XXIV	10.1	2.0	0.30	0.9	0.30	0.2	2.600
XXV	7.7	6.5	2.5	4.1	3.9	2.1	2.900
XXVI	6.0	5.0	1.6	1.0	0.9	2.5	1.760

^aD = diazepam, M = medazepam, III = oxazepam.

tion. Considerable difficulties were experienced in preparation of the starting 3-chloro derivatives IV-VI because of some misleading data found in the literature. A recently described⁵ procedure reported chlorination of 3-hydroxy compounds I and III with thionyl chloride on a steam bath. Although this procedure claimed to be a modification of some earlier ones,⁶⁻⁸ we obtained no isolable quantities of pure IV or VI. Careful tlc control (ether as eluent) in repeated experiments revealed that under these reaction conditions partial decomposition of the starting material occurred, while the isolation procedure caused hydrolysis of the products into the starting 3-hydroxy derivatives. We have found that the 3-hydroxy group in I-III can be quantitatively replaced by chlorine at a temperature under +5° using thionyl chloride containing catalytic quantities of dimethylformamide.^{10,11} Only free, analytically pure bases (IV-VI) were obtained after a simple isolation procedure; their structures were confirmed by nmr spectra.



	R ₁	R ₂	R ₃
I	CH ₃	H	OH
II	H	Cl	OH
III	H	H	OH
IV	CH ₃	H	Cl
V	H	Cl	Cl
VI	H	H	Cl

Biological Activity. Pharmacological screening of the compounds VII-XXVI revealed their useful biological properties (Table II). Until *in vitro* kinetic stability measurements are finished no conclusion can be drawn regarding what, if any, the contribution of the possible *in*

in vivo hydrolysis products, I-III, may be to the activity of the applied compounds. Methods used in the pharmacological tests have been repeatedly described in the literature.^{2,12,13}

Experimental Section

Melting points were determined on a Bøetius-Mikroheiztisch apparatus, 4°/min. Nmr spectra were taken on a Varian T-60 instrument in DMSO-*d*₆ using TMS as internal standard. Tlc was performed on coated plates (Merck Fertig Platten F 254).

General Procedure for IV-VI. Compounds I-III (0.1 mol, ~30 g) were added portionwise to 30 ml of freshly distilled thionyl chloride, which contained 0.5 ml of DMF. The reaction mixture was magnetically stirred and cooled to 0°. After the main quantity of I-III was added yellow crystals began to separate from the solution of V and VI, while IV remained in the solution. The mixture was left to stand on ice for 24 hr for IV and VI, and for 72 hr for V. The excess of thionyl chloride was evaporated *in vacuo* and traces were removed by repeated evaporation with dry benzene (5 × 10 ml).

Compound IV was isolated by addition of 140 ml of dry ether to the residual oily mass and stirred at ambient temperature. After several hours the separated crystals were filtered off from the pale-green mother liquor, repeatedly washed with ether, and dried to constant weight: yield 30.2 g (95%); mp 98-100°; nmr 3.42 (s, 3 H), 6.01 (s, 1 H), 7.3-7.9 ppm (m, 8 H). *Anal.* Calcd for C₁₆H₁₂Cl₂N₂O (319.2): C, 60.20; H, 3.79; N, 8.77. Found: C, 60.43; H, 3.67; N, 8.77.

Compound V was isolated as described for IV: mp 196-199° dec; yield 97%; nmr 6.12 (s, 1 H), 7.0-7.9 (m, 7 H), 11.3 ppm (s, 1 H). *Anal.* Calcd for C₁₅H₉Cl₃N₂O (339.6): C, 53.04; H, 2.67; N, 8.25. Found: C, 53.16; H, 2.43; N, 8.35.

Compound VI was isolated by treating the residual pale yellow crystals with 100 ml of absolute benzene at ambient temperature: yield 95.5%; mp 120-122°; nmr 6.10 (s, 1 H), 7.25-7.8 (m, 8 H), 11.35 ppm (s, 1 H). Traces of the 3-hydroxy compound gave rise to the small peaks at 4.96 (3-CH) and 11.0 (-OH) indicating high susceptibility of VI to the hydrolysis caused by the moisture present in DMSO-*d*₆. *Anal.* Calcd for C₁₅H₁₀Cl₂N₂O (305.1): C, 59.05; H, 3.30; N, 9.18. Found: C, 59.37; H, 3.12; N, 9.44.

General Procedure for Preparation of Compounds VII-XXX. 3-Chloro-1,4-benzodiazepin-2-ones IV-VI were dissolved in absolute acetonitrile, and a 0.5-10.0 molar excess of the requisite tertiary base was added. The reaction mixture was heated at 55-75° and magnetically stirred during 24-48 hr. All conversions were monitored following disappearance of the starting 3-chloro derivatives on tlc [benzene-acetone (2:1) as eluent]. Thereafter ether

was gradually added to the stirred cold solution until no more precipitation was observed. The resulting suspension of the amorphous crude product was stirred during several hours, and the product was filtered off. It was dissolved in hot methanol and filtered with charcoal, and filtrate was evaporated to a viscous oily residue. This was dissolved in warm acetone (or water) with stirring and set for crystallization, which started immediately or after prolonged chilling on ice in some cases.

To obtain compounds VII, VIII, X, XI, XIII, XIV, XIX, XXII, and XXVIII, the reaction temperature should not exceed room temperature. Strong decomposition of the material has been observed at higher temperatures. To obtain compounds XXIV, XXIX, and XXX reverse addition of the components and only 0.1–0.5 molar excess of the corresponding base were used at room temperature. To obtain compounds XVI–XVIII, XX, and XXV–XXVII, only a 0.1–0.3 molar excess of the corresponding base was used, and reaction temperature should be maintained at 55–75°. Purity of all products (VII–XXX) was controlled on tlc using acetone–acetic acid–water (12:1:1) as eluent, whereby sharp spots in the R_f range 0.3–0.8 have been obtained.

References

- N. Blažević, V. Šunjić, I. Crvelin, D. Kolbah, and F. Kajfež, *J. Heterocycl. Chem.*, **9**, 531 (1972).
- V. Šunjić, J. Kufinec, and F. Kajfež, *Arzneim.-Forsch.*, in press.
- S. J. Childress and M. I. Gluckman, *J. Pharm. Sci.*, **53**, 577 (1964).
- H. Klupp and J. Kähling, *Arzneim.-Forsch.*, **15**, 359 (1965).
- R. Y. Ning, W. Y. Chen, and L. H. Sternbach, *J. Org. Chem.*, **36**, 1067 (1971).
- S. C. Bell and S. J. Childress, *J. Org. Chem.*, **27**, 1691 (1962).
- S. C. Bell, R. J. McCaully, and S. J. Childress, *J. Org. Chem.*, **33**, 216 (1968).
- S. C. Bell, R. J. McCaully, C. Gochman, S. J. Childress, and M. I. Gluckman, *J. Med. Chem.*, **11**, 457 (1968).
- Belgium Patent 621,819; *Chem. Abstr.*, **60**, 2992h (1964).
- H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).
- K. Kikugawa and T. Kawashima, *Chem. Pharm. Bull.*, **10**, 2629 (1971).
- L. O. Randall, C. L. Schenckel, and R. F. Banziger, *Curr. Ther. Res., Clin. Exp.*, **590** (1965).
- M. I. Gluckman, *Curr. Ther. Res., Clin. Exp.*, **7**, 721 (1965).

Formation of Germine 3,15-Diacetate via the Isomerization of Germine 3,16-Diacetate

Edward M. Cohen, Rezso Aczel, MaryLou Torchiana,

Merck Sharp & Dohme Research Laboratories,
West Point, Pennsylvania 19846

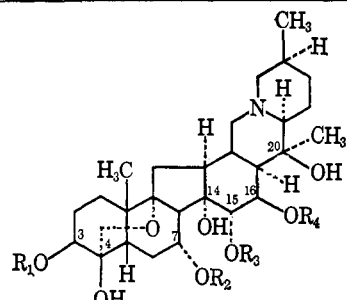
Roger Tull, and Edward J. J. Grabowski*

Merck Sharp & Dohme Research Laboratories,
Rahway, New Jersey 07065. Received April 16, 1973

Recent interest in various germine acetate esters has been prompted by their reported effectiveness in the treatment of myasthenia gravis.¹ Flacke and coworkers^{2a} have claimed the effectiveness of germine 3,16-diacetate (**1b**) in this area; however, this claim was subsequently modified when it was determined that germine 3,16-diacetate rapidly degraded in the aqueous solutions in which it was tested to afford a complex mixture of products. Among these was germine 3-acetate (**1c**), which was independently shown to be 40 times as active as germine 3,16-diacetate (**1b**) in a cat skeletal muscle assay.^{1b}

Two of us recently reported a detailed study of the stability of germine 3,16-diacetate in dilute aqueous solution.² On standing at its own pH (approximately 9.5) a 0.1% solution of **1b** rapidly ($t_{1/2} < 1$ hr) degrades to a mixture consisting of principally germine 3-acetate (**1c**)

Table I. Germine and Germine Esters



Compd	R ₁	R ₂	R ₃	R ₄	Acetyl methyl chemical shifts ^a
1a	H	H	H	H	
1b	CH ₃ CO	H	H	CH ₃ CO	1.98, 2.08
1c	CH ₃ CO	H	H	H	2.07
1d	H	H	H	CH ₃ CO	<i>b</i>
1e	CH ₃ CO	H	CH ₃ CO	H	2.08 (2CH ₃ CO-)
1f	CH ₃ CO	CH ₃ CO	CH ₃ CO	CH ₃ CO	2.00, 2.02, 2.05, 2.08
1g	H	H	CH ₃ CO	H	<i>d</i>

^aNmr chemical shifts in ppm (δ) relative to TMS in CDCl₃ solution. ^bSample insoluble in CDCl₃. ^cThe mass spectrum exhibited a molecular ion at m/e 593. ^dNot isolated.

and an unknown whose tlc R_f suggests that it is a diacetate. Minor components include germine 16-acetate (**1d**), a second unknown whose R_f suggests that it is a monoacetate, and germine (**1a**). In this communication we wish to report that the postulated² diacetate derived from **1b** is germine 3,15-diacetate (**1e**), a hitherto unreported germine acetate, and that it is comparable in activity to germine 3-acetate (**1c**).

In order to provide sufficient unknown diacetate for characterization and testing, we chose to carry out a preparative degradation of germine 3,16-diacetate (**1b**) in 50% aqueous pyridine. Qualitatively the degradation was identical with that reported for dilute aqueous solution,² but if offered a number of preparative advantages. Higher concentrations of **1b** could be used, thus obviating the need for large reaction volumes. More importantly, the half-life for germine 3,16-diacetate was increased to approximately 4 days, thus permitting termination of the degradation at a point most favorable to the isolation of the "new diacetate." After 21 days in this solution the germine 3,16-diacetate (**1b**) had almost completely degraded, but sufficient "unknown diacetate" remained for column chromatographic isolation. This was particularly important as the maximum tlc separation which could be achieved for **1b** and the "new diacetate" was approximately 0.05 R_f unit. Work-up of the degradation mixture followed by chromatography on silica gel afforded a chromatographically pure sample of the "unknown diacetate" as an amorphous solid. It was identical in tlc mobility with that obtained in minute quantity from the dilute aqueous degradation.² Hydrolysis in dilute aqueous solution afforded germine 3-acetate, germine, and a new component of the same R_f as that resulting from germine 16-acetate on hydrolysis, which has previously been tentatively identified as a monoacetate.² The nmr and mass spectra of the "unknown diacetate" confirmed that it was a new germine diacetate. Acetylation with acetic anhydride in pyridine afforded only germine 3,7,15,16-tetraacetate (**1f**).³ The new diacetate was stable to treatment with periodate under conditions used for previous structural