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.

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

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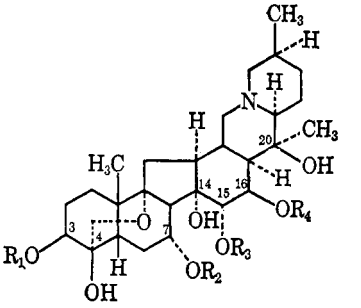
Recent interest in various germine acetate esters has been prompted by their reported effectiveness in the treatment of myasthenia gravis.[1] Flacke and coworkers[1a] have claimed the effectiveness of germine 3,16-diacetate (**1b**) in this area; however, this claim was subsequently modified when it 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**) 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 postulated[2] 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**).

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_3CO	H	H	CH_3CO	1.98, 2.08
1c	CH_3CO	H	H	H	2.07
1d	H	H	H	CH_3CO	*b*
1e[c]	CH_3CO	H	CH_3CO	H	2.08 ($2CH_3CO$–)
1f	CH_3CO	CH_3CO	CH_3CO	CH_3CO	2.00, 2.02, 2.05, 2.08
1g	H	H	CH_3CO	H	*d*

[a] Nmr chemical shifts in ppm (δ) relative to TMS in $CDCl_3$ solution. [b] Sample insoluble in $CDCl_3$. [c] The mass spectrum exhibited a molecular ion at m/e 593. [d] Not isolated.

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

Table II. Neuromuscular Activity of Germine Compounds

Compd[a]	IV dose,[b] mg/kg	N[c]	P/Po[d] Before dosing	P/Po[d] After dosing	Δ[e]	Rel potency[f]
1a	20	4	0.19 (0.08)	0.55 (0.15)	0.36	~0.05
1d	5[g]					
1b	40	4	0.29 (0.09)	0.75 (0.07)	0.46	~0.025
1c	1	4	0.20 (0.05)	0.70 (0.18)	0.50	1
1e	1	3	0.41 (0.08)	0.92 (0.28)	0.51	1

[a]See Table I. [b]Compounds dissolved in water and solution adjusted to pH 5 with HCl. [c]Number of cats per dose. [d]Ratio of twitch tension (*P*) to tetanic tension (*Po*) of gastrocnemius–soleus muscle in anesthetized cats; average value reported for tests with standard deviation given in parentheses. [e]Difference of average tension responses before and after dosing. [f]**1c** (germine 3-acetate) = 1. [g]Insufficient material for dose–response test; compound inactive at 1.0 and 5.0 mg/kg iv dose.

work in the germine series.[4] As a control, a mixture rich in all of the germine 3,16-diacetate degradation components was subject to treatment with periodate, and only the new diacetate proved to be inert. The only possible diacetate consistent with acylation to tetraacetate **1f**, yet inert to periodate, thus, lacking a vicinal diol system, is germine 3,15-diacetate (**1e**). The amorphous diester was converted to its crystalline oxalate salt for analysis and testing.

As previously noted[2] the second unknown component formed in the degradation of germine 3,16-diacetate (**1b**) appeared to be a monoacetate by tlc R_f. It is also generated from germine 16-acetate.[2] We have shown by comparative tlc that it is also generated *via* the degradation of germine 3,15-diacetate (**1e**) in aqueous solution. This suggests that the final unknown component formed in the aqueous degradation of **1b**, **1d**, and **1e** is germine 15-acetate (**1g**). No effort has been made to isolate and further characterize this compound (Table I).

The 16 to 15 transacylation which occurs in **1b** and probably in **1d** has proven to be an unusually facile reaction. Qualitatively it occurs at a rate comparable to the hydrolysis at 16, which is known to be intramolecularly catalyzed by the nitrogen present in the EF ring juncture.[5] In nonaqueous systems (methylene chloride, chloroform, acetonitrile, acetone, and pyridine) **1b** is stable,[2] and only in the presence of water does the transacylation occur. Presently, we see no strong driving force which explains the facility of the isomerization. However, there is sufficient flexibility in the ring system to permit interaction between positions 15 and 16.

Biological Data. The compound was tested relative to its ability to affect tension responses of the gastrocnemius and soleus muscles to stimulation of the sciatic nerve in anesthetized cats.[6] For comparison, data on all of the discussed germine esters are reported. Germine 3,15-diacetate was shown to be comparable in activity to germine 3-acetate under conditions in which negligible degradation had occurred. Both the 3-acetate and the 3,15-diacetate are 40 times as active as germine 3,16-diacetate. The data are reported in Table II.

Experimental Section

All the work was done using silica gel plates employing a freshly prepared solution of ethyl acetate–methanol–concentrated ammonia (80:15:5) for elution.[2] The approximate R_f's of the six components formed in the degradation of **1b** are: germine 3,15-diacetate (**1e**), 0.80; germine 3,16-diacetate (**1b**), 0.75; germine 3-acetate (**1c**), 0.60; germine 15-acetate (**1g**), 0.45; germine 16-acetate (**1d**), 0.40; germine, 0.25.

The periodate experiments were performed as previously described[4] and analyzed by tlc as indicated above. The acylation to form the tetraacetate from germine 3,15-diacetate was run as described by Kupchan[3] for the preparation from germine, and a 75% yield of **1b** was obtained, identical with authentic material prepared from germine.

Germine 3,15-Diacetate (1e). A solution of 10 g of germine 3,16-diacetate (**1b**) in 100 ml of pyridine and 100 ml of water was allowed to stand for 3 weeks at room temperature. The reaction solution was monitored by tlc as indicated above. After the 3-week period most of the germine 3,16-diacetate had degraded, but substantial germine 3,15-diacetate remained. After addition of 50 ml of concentrated NH_4OH the reaction solution was extracted with 3 × 100 ml of $CHCl_3$, which was dried over $MgSO_4$, filtered, concentrated at reduced pressure, and dried at 100° (vacuum) to afford 8.1 g of a colorless glass. Tlc examination (as above) indicated the presence of germine 3,15-diacetate, germine 3-acetate, and germine as the principal components. Dry column chromatography of 1 g of the product mixture on 200 g of Brinkmann silica gel H using 10% EtOH in $CHCl_3$ as the eluent, and taking 10-ml fractions, afforded essentially single-spot germine 3,15-diacetate in fractions 91–150. These were combined and concentrated to afford 0.14 g of **1b** as an amorphous powder whose chemical and spectral properties were as described in the text. Repeated attempts to crystallize the free base were not successful. To this material in methanol was added 1 molar equiv of oxalic acid. Concentration and trituration of the residual gum with CH_3CN afforded 139 mg of germine 3,15-diacetate oxalate monohydrate: mp 214–216° dec. *Anal.* ($C_{33}H_{15}NO_{15}$) C, H, N.

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Polymeric Salicylate Derivatives

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The attachment of biologically active compounds to polymers has been recently investigated as a means of increasing their duration of activity.[1-12] In the present work we have studied the attachment of salicylates, which are important as analgetics and antiinflammatory agents by carbonate linkages to starch. These linkages can suffer chemical and enzymatic hydrolysis releasing the active compound in the body.

Some work on the incorporation of salicylates into polymers has been carried out.[13-19] Thus, *O*-acetylsalicyloyl chloride was allowed to react with soluble starch in the presence of potassium hydroxide, and a polymer having 0.7 aspirin groups per glucose unit was obtained.[13-14]