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NEOGERMINE 7,9,14-ORTHOESTERS†: SYNTHESIS AND USE IN THE PREPARATION OF HYPOTENSIVE GERMINE 3,7,15-TRIESTERS.

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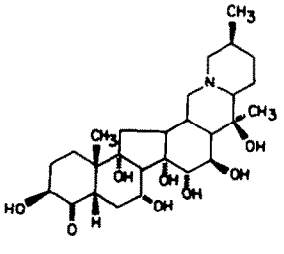
Abstract: Reaction of germine with $CH_3C(OEt)_3$/p-TsOH in DMSO at room temperature gave neogermine 7,9,14-orthoacetate (5). Treatment of germine 3,16-diacetate with $CH_3C(OEt)_3$ or triethyl orthoisobutyrate under the same conditions gave the corresponding 7,9,14-orthoacetate (6) and 7,9,14-orthoisobutyrate (9) respectively. Acid hydrolysis of these compounds led to conversion of the orthoester group to a 15-ester. If the 15-OH group was first esterified, then acid hydrolysis converted the orthoester to a 7-ester. The utility of this route for the synthesis of hypotensive germine 3,7,15-triesters is demonstrated by the preparation of known (14) and novel (16,17) derivatives.

INTRODUCTION

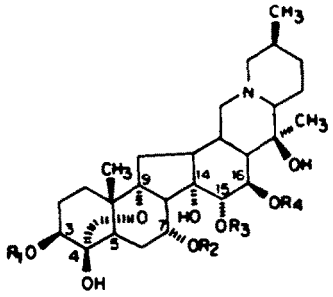
Attempts to use Veratrum alkaloids for the control of hypertension have been made since the middle of the 19th century. The crude extracts available then gave erratic results and it was not until the 1940's that the first crystalline alkaloid preparation, protoveratrine, became available and was shown to be suitable for clinical use in certain types of hypertension. The major limitation of this class of compounds is the powerful emetic effect that occurs close to the effective antihypertensive dose. This narrow therapeutic ratio led to their disuse when other effective antihypertensive agents became available.[2]

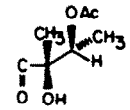
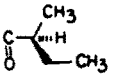
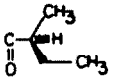
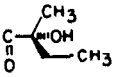
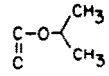
Extensive structural work[3] on these antihypertensive alkaloids, most notably by Kupchan, Barton, Prelog, Woodward, Jacobs, Fried and their co-workers, led to the isolation and identification of more than 20 alkaloids. All of these compounds were various esterfied forms of four alkamines, germine (1), protoverine, zygadenine and veracevine. The most potent antihypertensive agents[4] were derived from the first two, of which germitrine (3), germitetrine (2), neogermitrine and protoveratrine A and B proved to be useful. Extensive work by Kupchan et al[5] and also by Weisenborn et al[6] showed that the ester groups were extremely important for antihypertensive activity *viz* esters at C_3 and at C_{15} were essential for activity while an ester at C_7 (germine) or C_6 and C_7 (protoveratrine) was necessary for high potency with an acetate group being optimum. However none of the many, multiply esterified alkamines that were prepared showed a clearly improved therapeutic ratio when tested in conscious dogs.[4,7]

† Since the term germine refers strictly to a 5β-H, 3β-hydroxy alkaloid with a 4,9-hemiketal linkage we propose to use the trivial name neogermine for compounds that contain instead a C_4-ketone and a 9α-hydroxyl group, i. Derivatives of this alkaloid have not, heretofore, been described however, i is clearly implicated[3e] as an intermediate in the isomerisation of germine to isogermine (5α - epimer of i).



We considered the reflex mechanism[2b,4] by which the Veratrum alkaloids lower blood pressure to be quite desirable and therefore began a program aimed at the structural modification of the alkaloid nucleus itself in order to separate out the emetic effects. Since potential analogs would require appropriate esterification before biological evaluation we decided to investigate new methods for the selective introduction of these esters. We began this work with the readily available alkamine germine (1) and report here the preparation and use in this regard of the 7,9,14-orthoesters of germine.[8]



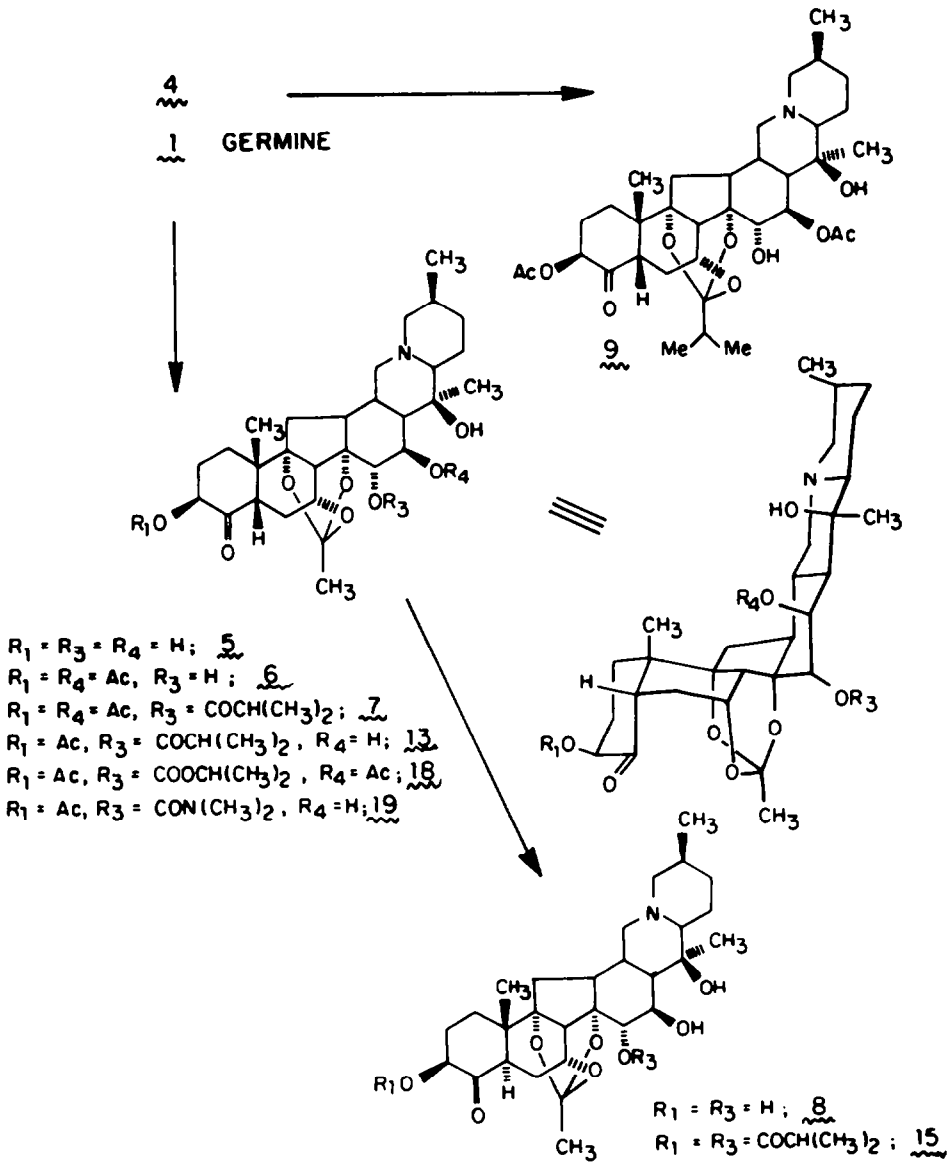
R_1	R_2	R_3	R_4		
H	H	H	H	;	1 (Germine)
	CCH_3 ‖ O		H	;	2
	CCH_3 ‖ O		H	;	3
CCH_3 ‖ O	H	H	CCH_3 ‖ O	;	4
CCH_3 ‖ O	H	CCH_3 ‖ O	CCH_3 ‖ O	;	10
CCH_3 ‖ O	CCH_3 ‖ O	CCH_3 ‖ O	CCH_3 ‖ O		11
CCH_3 ‖ O	H		H	;	12
CCH_3 ‖ O	CCH_3 ‖ O		H	.	14
CCH_3 ‖ O	CCH_3 ‖ O		H	;	16
CCH_3 ‖ O	CCH_3 ‖ O		H	.	17

RESULTS

Treatment of germine 3,16-diacetate[6] (4) with triethyl orthoacetate and p-TsOH in DMSO gave the 7,9,14-orthoacetate 6 in 74% crystalline yield. [TLC examination of the crude product indicated that no other products were formed in this reaction.] The assignment of the 7,9,14-orthoacetate structure to 6 follows from its' spectral data and from further chemical transforma-

tions. The ^{1}H-NMR spectrum of 6 clearly showed that the orthoacetate group was linked to three hydroxyl groups of germine since there were no signals evident for an OEt group. It's ^{13}C-NMR spectra[9] displayed an orthoester carbon resonance at 107 ppm and a ketone carbon resonance at 203 ppm; a ketone carbonyl absorption at 1720 cm^{-1} in the IR spectrum confirmed the presence of a ketone group. This showed that the hemiketal at C_4 had been converted to a ketone and that the orthoacetate was linked therefore to the 7α, 9α and 14α-hydroxyl groups. Further evidence for this was obtained by conversion of 6 to the 15-isobutyrate 7 with isobutyl chloride in pyridine whereby the 15β-H signal was shifted downfield by 1.32 ppm in the ^{1}H-NMR spectrum. Evidence that epimerisation at C_5 to the more stable trans ring junction had not occurred under the reaction conditions, was obtained from the ^{1}H-NMR spectrum of 6 which showed an equatorial 3α-H at 5.05 ppm. Also treatment of 6 with base to induce epimerisation at C_5 gave ketone 8 which showed an axial 5α-H [3.18 ppm dd, J 12,4 Hz)] as well as an axial 3α-H [4.18 ppm (m, w 1/2 10 Hz)] in it's ^{1}H-NMR spectrum. This compound, isogermine 7,9,14-orthoacetate (8) was further characterized as the 3,15-disobutyrate (15). Further evidence for structure 6 follows from its conversion to germine 3,7,15,16-tetraacetate 11 *vide infra.*

SCHEME 1

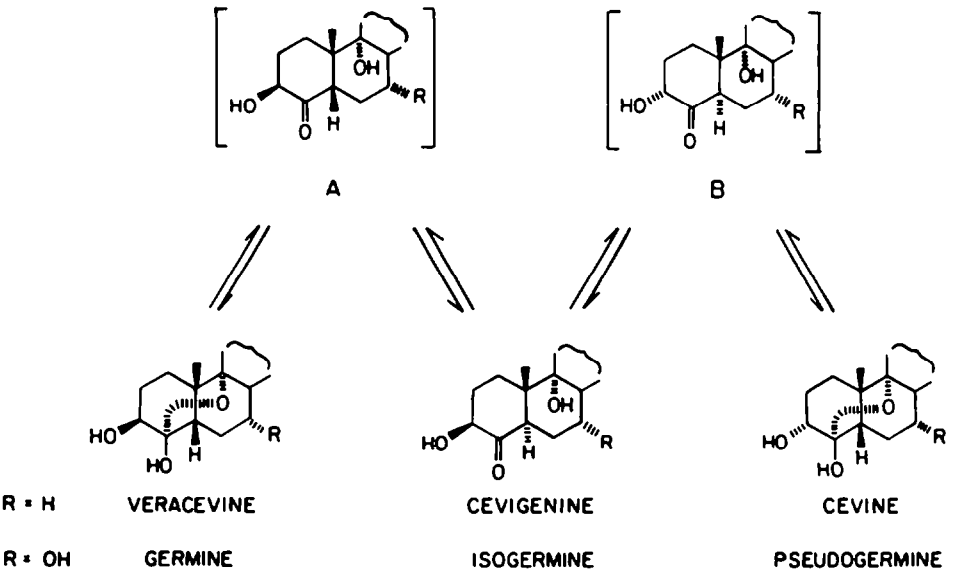


Treatment of germine (1) with triethylorthoacetate under these conditions gave a complex mixture (TLC, NMR) from which neogermine 7,9,14-orthoacetate (5) crystallised out. This single reaction gives in up to 45% yield a product whereby three of the seven hydroxyl groups of germine have been differentiated and protected. As will be shown later, this protecting group is also a latent 7-acetate group which is often a difficult ester to form selectively. Treatment of 5 with base gave the isogermine derivative 8 identical to that produced from 6 above. Reaction of germine 3,16-diacetate (4) with the more sterically demanding triethyl orthoisobutyrate also gave the desired 7,9,14-orthoisobutyrate 9 albeit in a somewhat lower yield (40%).

Hydrolysis of the 7,9,14-orthoacetate group of 6 with dilute aqueous AcOH at room temperature gave germine 3,15,16-triacetate 10 as an amorphous solid in 62% yield. Acetylation (Ac_2O/Py) of 10 then gave the 3,7,15,16-tetracetate[10] 11, identical (TLC, NMR) with that produced by direct acetylation of germine (1). This conversion shows that the original stereochemistry at C_3 and C_5 was maintained through both the orthoester forming and the acid hydrolysis steps. Methanolysis of 9, to remove the acetate at C_{16}, followed by acid hydrolysis of the 7,9,14-orthoisobutyrate gave the 15-isobutyrate 12. In both cases it was clear that acid hydrolysis gave the 15-ester from the position of the geminal 15β-H in the ^{1}H-NMR spectra *viz* 10 5.25 ppm (d,J5Hz) and 12 5.30 ppm (d,J5Hz). However, if the 15α-hydroxyl is blocked then acid hydrolysis of the 7,9,14-orthoester gives the 7-ester. Thus methanolysis of 7 to give 13 followed by acid hydrolysis gave germine 3,7-diacetate 15-isobutyrate (14).

In a preparative fashion the 7,9,14-orthoester group can be used in the synthesis of desired germine di and triesters. It first functions as a protecting group for the 7α and 14α -hydroxyl groups and is then convertable to a 15-ester (as in the preparation of 12) or a 7-ester (preparation of 14). The synthesis of the novel triesters 16 and 17 provide further examples of the utility of this method. Thus treatment of 6 with phosgene followed by quenching with 2-propanol gave the 15α-isopropylcarbonate 18. Methanolysis, to remove the 16β-acetate followed by acid hydrolysis then gave the desired triester 16 in 27% overall yield. Substitution of dimethylamine for isopropanol in this sequence gave the 15α-dimethylcarbamate analog 17[12].

The A, B ring system of germine and related alkaloids is notoriously unstable to base treatment which lead to some confusion in the early days of the structural elucidation of these alkaloids. Barton, Jeger, Prelog and Woodward[3e] accounted for the experimental facts by proposing the following sequence for the veracevine-cevigenine-cevine isomerisation (R=H) which is accepted today also for germine (R=OH) and the other related alkaloids.

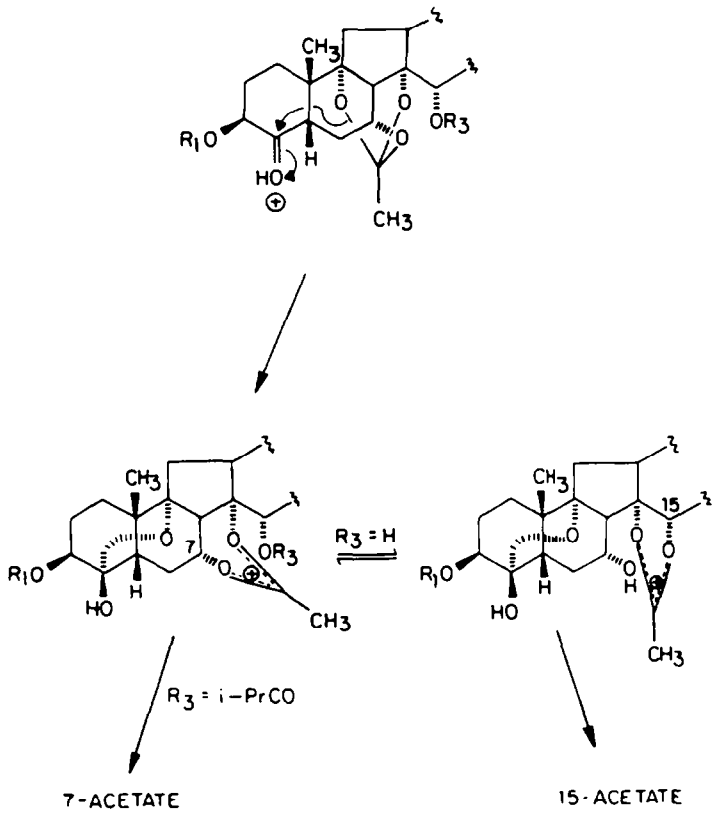


The driving force for this isomerisation is the conversion of an axial hydroxyl group at C_3 to an equatorial position (3β-OH in the iso series and 3α -OH in the pseudo series). However, neither of the intermediates in these transformations, A and B, nor any of their derivatives have been prepared to date. The 7,9,14-orthoesters described here are therefore the first examples of the intermediate structure type A to be reported. From the reactions adumbrated above the A, B cis ring fusion in these derivatives is stable to strong acid (p-TsOH) and weak organic bases (pyridine) but not to strong base (NaOMe) at room temperature.

Since germine (1) on treatment with acetone under acid conditions gave exclusively the 14,15-acetonide[13] we were somewhat surprised to find that the exchange reaction with triethyl orthoacetate under these conditions gave the 7,9,14-orthoester. Presumably in each reaction the end product reflects the thermodynamically most stable product and therefore that, in the case of orthoester formation, the gain in forming a trioxobicyclo [2,2,2] octane structure[14] more than compensates for the breaking of the hemiketal linkage. Acetonide formation between the 7α and 14α-hydroxyl groups does not have this stabilizing influence available to it but instead would be disfavoured by non-bounded steric interactions between one of the methyl groups of the gem dimethyl group and the 15β-hydrogen.

A reasonable mechanism for the acid hydrolysis of these orthoesters is shown in Scheme 2. Initial protonation of the C_4-ketone followed by reformation of the 4,9-hemiketal linkage generates a 7,14-dioxolenium cation. Isomerisation to a 14,15-dioxolenium cation followed by attack of water then gives the least sterically hindered 15-ester. If, however, the 15α-hydroxyl is already esterified then the 7,14-dioxolenium cation cannot isomerise and hydration yields the 7-ester. That the formation of the C_4 hemiketal provides a major driving force for this orthoester hydrolysis is shown by the complete stability of the isogermine 7,9,14-orthoacetate (15) to strong acid conditions (4N HCl/3H/RT)[15]. The trans A,B ring junction of 15 of course, precludes hemiketal formation.

SCHEME 2



Acknowledgment

We would like to thank Dr. John G. Topliss for encouragement and stimulating discussions during this work. We also thank Dr. B. Pramenik for the high resolution FAB mass spectral data and the staff of Analytical Research Services for the spectral and microanalytical data.

EXPERIMENTAL

Melting points were taken on a Fisher Digital melting point analyzer Model 355 and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on Varian CFT-20 and XLFT-100 instruments, respectively, in $CDCl_3$ solution with Me_4Si as an internal standard. Optical rotations were determined at 25°C as pyridine solutions. IR spectra were recorded on a Perkin-Elmer 180 infrared grating spectrophotometer as nujol (νcm^{-1}). Medium resolution mass spectra were taken on a Varian MAT CH5 spectrometer using a 70-eV source. Fast Atom Bombardment (FAB) mass spectra were obtained on a Finnigan MAT 312 double focussing mass spectrometer operating at an accelerating voltage of 3KV; samples were ionized by bombardment with xenon atoms produced by a saddle field ion source from ion tech, operating with a tube current of 2mA at an energy of 7KeV. The composition of the adduct ion corresponding to $(M + H)^+$ was obtained by peak matching. Silica gel preparative (2000 μm) and analytical (250 μm) thin-layer chromatography (TLC) plates were obtained from Analtech, Inc., and the silica gel used for column chromatography was TLC grade supplied by E. Merck (silica gel G-60).

Neogermine 3,16-diacetate 7,9,14-orthoacetate (6). Germine 3,16-diacetate[11] (4,3.0g, 5.05 mmol) was dissolved in a mixture of triethyl orthoacetate (17 mL, 92.7 mmol), Me_2SO (17 mL) and p-$TsOH.H_2O$ (1.5 g, 7.9 mmol) and left at room temperature for 2 h. The reaction mixture was diluted with $CHCl_3$ (75 mL) and washed with dilute NH_4OH. The aqueous layer was extracted with $CHCl_3$ and the combined organic extracts were washed twice with H_2O, dried ($MgSO_4$) and concentrated to an oil under reduced pressure. This residue crystallised from hexane-$CHCl_3$ to give 6 (2.3g, 74%): mp 219-220 °C; $[\alpha]_D$-83.0° (c,0·74); IR 1740, 1720; ^{1}H-NMR 5.70 (1H, d, J=4 Hz, H16), 5.05 (1H,M, H_3), 4.28 (1H, M H_7), 3.92 (1H, d, J=4Hz, H_{15}), 2.10 (3H, s, $OCOCH_3$), 1.98 (3H, s, $OCOCH_3$), 1.29 (3H, s, orthoester CH_3), 1.26 (3H, s, H_{21}), 1.20 (3H, s, H_{19}), 1.05 (3H, d, J = 8 Hz, H_{27}); ^{13}C NMR 203.1 (s, C_4) 169.8, 168.8 (s,$OCOCH_3$), 106.9 (s,orthoester C), 86.2 (s,C_9), 82.2 (s,C_{14}), 74.24 (d,C_3), 71.0, 70.5, 69.5, 67.8, 66.1 (C_7, C_{15}, C_{16}, C_{20}, C_{22}) 61.8, 61.4 (t,C_{18}, t,C_{26}), 49.1 (d,C_5), 44.0, 43.9, 41.4 (C_{10}, C_{12}, C_{17}), 37.2, 36.6, 34.7 (C_8, C_{11}, C_{13}), 29.9, 29.0, 27.4, 25.6, 25.3, 24.4, 23.4, 21.4, 21.0 (C_1, C_2, C_6, C_{21}, $C_{23\text{-}25}$, $OCO\underline{C}H_3$), 20.2 (q,$C\underline{C}H_3$), 18.5 (q,C_{19}), 17.1 (q C_{27}); MS, m/e 617 (M^+,8), 112 (100). Anal. Calcd. for $C_{33}H_{47}O_{10}N$: C, 64.16; H, 7.67; N, 2.27. Found: C, 64.06; H, 7.60; N, 1.95.

Neogermine 7,9,14-orthoacetate (5). Germine[16] (1,20 g, 39.2 mmol) and p-$TsOH.H_2O$ (11.5g, 60 mmol) were dissolved in Me_2SO (50 mL) and triethylorthoacetate (50 mL, 0.27 mol) at room temperature. After 0.3h the reaction was cooled to 0° and H_2O (8 mL) was added with efficient stirring. After 2 min., the reaction mixture was diluted with $CHCl_3$ (200 mL) and washed with dilute NH_4OH. The aqueous layer was extracted with $CHCl_3$ (2 x 100 mL) and the combined organic solutions were washed with H_2O (3 x 100 mL), dried ($MgSO_4$) and evaporated. Crystallisation of the residue from CH_2Cl_2 (80 mL) gave 5 (9.7g, 48%) as a CH_2Cl_2 solvate: mp 168-175 °C; $[\alpha]_D$-62.9° (c,0.91); IR 1700; ^{1}H NMR 4.44 (2H, m, H_{16} and H_7), 4.00 (2H, m, H_{15} and H_3), 1.26 (9H, s, H_{19}, H_{21} and orthoester CH_3), 1.07 (3H, d, J = 7 Hz, H_{27}); ^{13}C NMR 210.7 (C_4), 106.8 (orthoester C), 86.2 (C_9), 82.5 (C_{14}), 73.3, 73.0 (C_3, C_{20}), 70.2, 69.7, 68.6, 66.4 (C_7, C_{15}, C_{16}, C_{22}), 61.4, 61.1 (C_{18}, C_{26}), 47.9 (C_5), 44.3, 43.5, 41.0 (C_{10}, C_{12}, C_{17}), 37.2, 36.5, 33.8 (C_8, C_{11}, C_{13}), 29.4, 28.9, 27.4, 26.4, 25.4, 24.5, 23.6 (C_1, C_2, C_6, C_{21}, $C_{23\text{-}25}$), 20.1 (CCH_3), 18.4 (C_{19}), 17.1 (C_{27}); MS, m/e 533 (M^+, 15), 112 (100); FAB-MS, m/e calcd. for $C_{29}H_{44}O_8N$ $(M+H)^+$534.3066, found 534.3025. Anal. Calcd. for $C_{29}H_{43}O_8N$. 1/4 mol CH_2Cl_2: C, 63.31; H, 7.90; N, 2.52 Found: C, 62.02; H, 7.99; N, 2.45.

Neogermine 3,16-diacetate 7,9,14-orthoisobutyrate (9). A solution of germine 3,16-diacetate (4, 1.0g, 1.68 mmol) and p-TsOH.H_2O (0.48g, 2.52 mmol) in Me_2SO (6 mL) and triethyl orthoisobutyrate (3 mL) was left at room temperature for 0.5 h. H_2O (1 mL) was added with stirring and the reaction mixture was diluted with $CHCl_3$ (30 mL) and washed with dilute NH_4OH. The aqueous layer was washed with H_2O (3 x 30 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed over silica gel (120g) and elution with $CHCl_3$-MeOH-NH_4OH (700:10:1) gave 9 (0.43g, 40%) as a amorphous solid: $[\alpha]_D$-72.9 (C, 0.56); ^{1}H-NMR 5.68 (1H, d, J = 4 Hz, H_{16}), 4.98 (1H, m, H_3), 4.28 (1H, m, H_7), 3.90 (1H, d, J = 4,Hz H_{15}), 2.10 (3H, s, $OCOCH_3$), 1.98 (3H, s, $OCOCH_3$), 1.25 (3H, s, H_{21}), 1.20 (3H, s, H_{19}), 1.05 (3H, d, J=7Hz, H_{27}), 0.83 (6H, J=7 Hz, $CH(CH_3)_2$); MS, m/e 645 (M^+,16), 602 (12), 586 (10); FAB-MS, m/e calcd. for $C_{35}H_{52}O_{10}N$ $(M + H)^+$ 646.3590, found 646.3511.

Neogermine 3,16-diacetate 15-isobutyrate 7,9,14-orthoacetate (7). Neogermine 3,16-diacetate 7,9,14-orthoacetate (6, 0.5g, 0.81 mmol) in pyridine (2.5 mL) was stirred at room temperature with isobutyric anhydride (0.3 mL) for 24 h. A further portion of isobutyric anhydride (0.3 mL) and pyridine (0.5 mL) was added and after 24 h. the reaction mixture was cooled to 0°C and MeOH (1 mL) was added. After 3 min. the solvents were removed under reduced pressure and the residue was taken up in $CHCl_3$, washed with dilute NH_4OH, dried ($MgSO_4$) and evaporated. Crystallisation of the residue from Et_2O/hexane gave 7; mp 180-181 °C; $[\alpha]_D$-76.9° (c, 1.03); IR 1745, 1720; ^{1}H-NMR 5.50 (1H, d, J= 4 Hz, H_{16}), 5.24 (1H, d, J = 4 H_{15}), 5.00 (1H, m, H_3), 4.38 (1H, m, H_7), 2.07 (3H, s, $OCOCH_3$), 1.98 (3H, s, $OCOCH_3$), MS, m/e 687 (M^+,9). Anal. Calcd. for $C_{37}H_{53}O_{11}N$: C, 64.61; H, 7.77; N, 2.04. Found: C, 64.92; H, 8.00; N, 1.84.

Isogermine 7,9,14-orthoacetate (8). Neogermine 7,9,14-orthoacetate 5 (0.8g, 1.5 mmol) and NaOMe (0.4g, 7.4 mmol) were dissolved in MeOH (10 mL) and stirred under N_2 at room temperature for 17 h. After removal of the solvent under reduced pressure, the residue was taken up in dilute NH_4OH and extracted into $CHCl_3$ (3 x 30 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to a solid residue (0.62g) which was chromatographed over silica gel (60g). Elution with $CHCl_3$-MeOH-NH_4OH (200:10:1) gave 8 (0.32g, 40%) which crystallised from CH_2Cl_2-hexane as a CH_2Cl_2 solvate (0.25g): mp 266-267 °C; $[\alpha]_D$-54.8° (c, 0.66) IR 1720; ^{1}H-NMR 4.45 (2H, m, H_7 and H_{16}), 4.18 (1H, m w1/2=10Hz, H_3), 4.02 (1H, d, J=4Hz), 3.20 (1H, dd, J=12,4Hz, H_5), 1.48 (3H, s, orthoester CH_3), 1.25 (3H, s, H_{21}), 1.05 (3H, d, J = 8 Hz, H_{27}), 0.82 (3H, s, H_{19}); MS, m/e 533 (M^+, 15), 112 (100). Treatment of germine 3,16-diacetate 7,9,14-orthoacetate (6) (50 mg, 0.09 mmol) with NaOMe (27 mg) in MeOH (0.75 mL) for 4 h at room temperature followed by work up as above gave 8 identical by ^{1}H NMR and MS with the sample produced from 5. Anal. Calcd. for $C_{29}H_{43}O_8N$.1/4 mol CH_2Cl_2: C, 63.31; H, 7.90; N, 2.52. Found: C, 63.30; H, 7.95; N, 2.37.

Isogermine 3,15-diisobutyrate 7,9,14-orthoacetate (15). A solution of isogermine 7,9,14-orthoacetate (8, 0.35g, 0.66 mmol) in pyridine (1.5 mL) was treated with isobutyryl chloride (0.19 mL, 1.8 mmol) for 3 h at room temperature. The solvent was evaporated under reduced pressure and the residue was taken up into $CHCl_3$, washed with dilute NH_4OH, dried ($MgSO_4$) and evaporated. The residue consisting of the desired 3,15-diisobutyrate admixed with the 3,15, 16-triisobutyrate was chromatographed on silica gel. Elution with $CHCl_3$-MeOH-NH_4OH (550:10:1) gave 15 (.095g, 20%) as an amorphous solid: $[\alpha]_D$-58.8 (c, 0.4); ^{1}H-NMR 5.40 (1H, d, J = 4Hz, H_{15}), 5.20 (1H, m, w1/2=20Hz, H_3); 4.55 (1H, m, H_7), 4.28 (1H, m, w1/2=6Hz, H_{16}), 3.25 (1H, dd, J=12,4Hz, H_5), 1.38 (3H, s, orthoester CH_3), 0.87 (3H, s, H_{19}); ^{13} CNMR 205.8 (C_4), 176.6, 175.4 ($O\underline{C}OCHMe_2$), 106.9 (orthoester C), 85.2(C_9), 79.7 (C_{14}), 73.0 (C_3), 69.6, 69.2 68.5, 65.4 (C_7, C_{15}, C_{16}, C_{20}, C_{22}), 61.4, 61.2 (C_{18}, C_{26}), 47.6 (C_5), 45.5, 45.2, 45.0 (C_{10}, C_{12}, C_{17}), 37.6, 37.2, 34.4, 34.1, 33.9 (C_8, C_{11}, C_{13}, $COCHMe_2$), 28.9, 28.7, 28.3, 27.3, 26.8, 23.9, 20.0, 19.2, 19.15, 19.0, 19.95, 18.4 (C_1, C_2, C_6, C_{21}, C_{23-25}, $C\underline{C}H_3$, $COCH(\underline{C}H_3)_2$); MS, m/e 673 (M^+,21). Anal. Calcd. for $C_{37}H_{55}O_{10}N$: C, 65.95; H, 8.23; N, 2.08. Found C, 65.91; H, 8.39; N, 1.92.

Germine 3,15,16-triacetate (10). Neogermine 3,16-diacetate 7,9,14-orthoacetate (6, 0.5g, 0.8 mmol) was dissolved in AcOH (3 mL) and H_2O (10 mL). After 23 h at room temperature the reaction mixture was neutralized with dilute NH_4OH and the product was extracted into $CHCl_3$ (3 x 50 mL). The combined organic solutions were dried ($MgSO_4$), and evaporated to a residue (0.52g) which was chromatographed over silica gel (50g). Elution with $CHCl_3$-MeOH-NH_4OH (500:10:1) gave 10 (0.32g, 62%) as an amorphous solid: $[\alpha]_D$-21.2 (c, 0.57); IR 1740; ^{1}H-NMR 5.56 (1H, d, J = 4 Hz, H_{16}), 5.25 (1H, d, J = 4 Hz, H_{15}), 4.90 (1H, m, H_3), 4.42 (1H, m, H_7), 2.09 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 1.97 (3H, s, $OCOCH_3$), 1.17 (3H, s, H_{21}), 1.05 (3H, d, J = 7 Hz, H_{27}), 1.00 (3H, s, H_{19}); MS, m/e 635 (M^+,3.8), 112 (100). Anal. Calcd. for $C_{33}H_{49}O_{11}N$: C, 62.34; H, 7.77; N, 2.20. Found: C, 61.84; M, 7.93; N, 1.95.

Germine 3,7,15,16-tetracetate (11). A solution of germine 3,15,16-triacetate (10, 0.21g, 0.33 mmol) in pyridine (0.5 mL) and Ac_2O (0.25 mL) was left at room temperature for 17 h. The reaction mixture was cooled in an ice bath and MeOH (0.2 ml) was added with stirring. After 5 min. the solvents were evaporated, the residue was taken into $CHCl_3$, washed with dilute NH_4OH, dried ($MgSO_4$) and evaporated to give 11. The ^{1}H-NMR spectrum of 11 was identical with that of an authentic sample derived from the direct acetylation of germine[11].

Germine 3-acetate 15-isobutyrate (12). Neogermine 3,16-diacetate 7,9,14-orthoisobutyrate (9, 0.35g, 0.51 mmol) was dissolved in MeOH (10 mL) and left at room temperature for 44 h. Evaporation of the solvent and chromatography of the residue over silica gel gave, on elution with $CHCl_3$-MeOH-NH_4OH (700:10:1) germine 3-acetate 7,9,14-orthoisobutyrate (0.22g, 68%) as an amorphous solid: $[\alpha]_D$-62.4 (c, 0.38); IR 1740, 1720; ^{1}H-NMR 4.95 (1H, m, H_3), 4.42 (2H, m, H_{16} and H_7), 3.98 (1H, d, J = 4 Hz, H_{15}), 2.05 (3H, s, $OCOCH_3$), 1.22 (3H, s, H_{21}), 1.20 (3H, s, H_{19}), 1.05 (3H, d, J = 7 Hz, H_{27}); MS, m/e 603 (M^+,11), 112 (100). A portion of this material (0.13g, 0.22 mmol) was dissolved in AcOH (1 mL) and H_2O (3 mL) at room temperature. After 17 h dilute NH_4OH was added and the product extracted into $CHCl_3$ (2 x 50 mL). The combined extracts were dried ($MgSO_4$) and evaporated to a residue which crystallised from hexane-$CHCl_3$ to give 12 (0.055g, 41%). Purification of the mother liquors on preparative layer silica gel chromatography (development solvent; $CHCl_3$-Me_2CO; 1:1) gave additional 12 (0.040g, total yield 0.095g, 71%): mp 245-248 °C; $[\alpha]_D$-9.6° (c, 0.34) ^{1}H-NMR, 5.30 (d, J=4 Hz, H_{15}), 4.90 (1H,m, H_3), 4.58 (1H,m, H_7), 4.25 (1H,m, w1/2=7 Hz, H_{16}), 2.05 (3H,s, $OCOCH_3$), 1.20 (3H,s, H_{21}), 1.12 (6H,d, J = 7 Hz, $CH(CH_3)_2$), 1.05 (3H,d, J=7Hz, H_{27}), 0.95 (3H,s, H_{19}); MS, m/e 621 (M^+,4). Anal. Calcd. for $C_{33}H_{51}O_{10}N$: C, 63.75; M, 8.27; N, 2.25. Found: C, 63.87; H, 8.04; N, 2.11.

Germine 3,7-diacetate 15-isobutyrate (14). A solution of 7 (2.5g, 3.64 mmol) was dissolved in MeOH (100 mL) for 43 h at room temperature. Evaporation of the solvent and chromatography of the residue over silica gel, eluting with $CHCl_3$-MeOH-NH_4OH (250:10:1), gave germine 3-acetate 15-isobutyrate 7,9,14-orthoacetate 13 (1.3g, 55%) as an amorphous solid: $[\alpha]_D$-66.3 (c, 0.73); ^{1}H-NMR 5.35 (1H, J = 4 Hz, H_{15}), 5.01 (1H,m,H_3), 4.48 (1H, m,H_7), 4.20 (1H, m, H_{16}), 2.06 (3H, s,$OCOCH_3$), 1.22, 1.20, 1.18, 1.10, 1.05, (15H, H_{21}, H_{19}, H_{27}, $CH(CH_3)_2$); m/e calcd. for $C_{35}H_{52}O_{10}N$ $(M + H)^+$ 646.3590, found 646.3524. A portion of this material (0.3g, 0.47 mmol) was dissolved in 4N HCl (12 mL) for 25 min at room temperature. The reaction mixture was neutralized with dilute NH_4OH and the product extracted into $CHCl_3$ (2x 50 mL), the combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to a solid residue. Crystallisation from hexane-$CHCl_3$ gave 14 (0.16g, 52%): mp 228-233 °C (dec.); $[\alpha]_D$-80.0 (c,0.79); ^{1}H-NMR 5.78 (1H,m, H_7), 5.15 (1H, d, J = 4 Hz, H_{15}), 4.95 (1H, m, H_3), 4.22 (1H, m, w1/20=6Hz, H_{16}), 2.08 (3H, s, $OCOCH_3$), 2.05 (3H,s, $OCOCH_3$), 1.18 (3H, s, H_{21}), 1.14 and 1.12 (6H, $CH(CH_3)_2$), 1.05 (3H, d, J = 7 Hz, H_{27}), 0.98 (3H, s, H_{19}); MS, m/e 663 (M^+, 20), 604 (46). Anal. calcd. for $C_{35}H_{53}O_{11}N$: C, 63.33; H, 8.05; N, 2.11. Found: C, 63.10; H, 8.08; N, 1.84.

Germine 3,7-diacetate 15-isopropylcarbonate (16). To a solution of neogermine 3,16,-diacetate 7,9,14-orthoacetate (6, 2.0g, 3.24 mmol) in pyridine (10 mL) cooled in an ice-bath was added, dropwise, a 12.5% solution of $COCl_2$ in benzene (3.2 mL, 3.24 mmol). The reaction mixture was brought to room temperature, stirred for 2 h and treated with isopropanol (0.4 mL). After a further 0.5 h the solvents were evaporated, the residue taken into $CHCl_3$ and washed with dilute NH_4OH, dried ($MgSO_4$) and evaporated. The residue was dissolved in MeOH (30 mL) and left at room temperature for 24 h. After removal of the solvent the residue was dissolved in 4N HCl (30 mL) and left at room temperature for 0.5 h. $CHCl_3$ was added and the mixture was neutralized with dilute NH_4OH; the aqueous layer was extracted with $CHCl_3$ (2 x 50 mL) and the combined $CHCl_3$ solutions were dried ($MgSO_4$) and evaporated. Chromatography of the residue over silica gel gave, on elution with $CHCl_3$-MeOH-NH_4OH (550:10:1), 16 (0.60g, 27%). Crystallisation from hexane-CH_2Cl_2 gave the analytical sample: mp 223-226 °C; $[\alpha]$-57.1° (c, 0.80); ^{1}H-NMR 5.77 (1H, m, H_7), 4.95 (2H, m, H_3 and H_{15}), 4.80 (1H, sept., J = 6 Hz, $OCHMe_2$), 2.08 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 1.26 (6H, d, J=6Hz $CH(CH_3)_2$) 1.18 (3H, s, H_{21}), 1.03 (3H, d, J = 7 Hz, H_{27}), 0.98 (3H, s, H_{19}); MS, m/e 679 (M^+,8), 620 (18). Anal. calcd. for $C_{35}H_{53}O_{12}N$: C, 61.84; H, 7.86; N, 2.06. Found: C, 61.79; H, 8.17; N, 2.02

Germine 3,7-diacetate 15-N,N-dimethylcarbamate (17) A 12.5% solution of $COCl_2$ in benzene (4 mL, 4.04 mmol) was added dropwise to a well stirred, cooled (ice-bath) solution of neogermine 3,16-diacetate 7,9,14-orthoacetate (6, 1.9g, 3.08 mmol) in pyridine (12 mL). The reaction mixture was brought to room temperature, stirred for 1 h and then cooled to 0° with ice bath. Dimethylamine was condensed into the reaction mixture at such a rate that the temperature did not rise above 25°C. After 0.5 h the solvents were evaporated and the residue was chromatographed over silica gel (200g). Elution with $CHCl_3$-MeOH-NH_4OH (270:10:1) gave neogermine 3,16-diacetate, 15-N, N-dimethylcarbamate 7,9,14-orthoacetate (0.4g, 19%) and the monoacetate 19 (1.2g 60%) as an amorphous solid: $[\alpha]_D$-50.6 (c, 0.64); ^{1}H NMR 5.17 (1H, d,J= 4Hz, H_{15}), 5.05 (1H, m,H_3), 4.45 (2H, m,H_7 and H_{16}), 2.88 (6H,s,$N(CH_3)_2$), 2.05 (3H, s,$OCOCH_3$), 1.20 (6H, s,H_{21} and orthoester CH_3), 1.17 (3H, s,H_{19}), 1.05 (3H, d,J = 8 Hz, H_{27}): MS, m/e 646 (M^+,39), 603 (45). 19 (0.12 g, 0.19 mmol) was dissolved in 4N HCl (2 mL), left at room temperature for 0.5 h and then brought to pH 9 by addition of dilute NH_4OH. The product was extracted into $CHCl_3$ (3 x 50 mL) and the combined extracts were dried ($MgSO_4$) and evaporated to a residue which crystallised on addition of CH_2Cl_2-hexane to give 17 (0.04g, 32%): mp 262-264 °C; $[\alpha]_D$-65.5° (c, 0.33); 1H-NMR, 5.77 (1H, m,H_7), 4.98 (2H, m,H_3 and H_{15}), 4.30 (1H, m,H_{16}), 2.86 (6H, s,$N(CH_3)_2$), 2.12 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 1.19 (3H, s,H_{21}), 1.05 (3H, d,J = 7 Hz, H_{27}), 0.98 (3H, s,H_{19}): MS, m/e 664 (M^+,12), 605 (24). Anal. Calcd. for $C_{34}H_{52}O_{11}N_2$: 1/2 mol H_2O: C, 60.61; H, 7.93; N, 4.16. Found: C, 60.63; H, 7.96; N, 4.09.

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