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Synthetic Hypotensive Esters from Germine[1]

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A number of synthetic esters possessing high hypotensive activity have been prepared by selective and stepwise esterification of germine. Evidence is presented indicating that direct acylation of germine introduces the acid radicals on the same hydroxyl groups found esterified in the natural di- and triesters. From the observed relationship of structure to activity, it appears that four- and five-membered α-branched acid radicals are necessary for appreciable hypotensive activity with the restriction that the over-all dimension of the germine ester molecule lies close to an optimum value.

In recent years extensive studies of the hypotensive constituents of a number of *Veratrum* species[3-9] and related plants[10] have shown that many of the active principles are esters of germine. Since germine is more easily obtained than any individual natural ester such as germitrine or neogermitrine, it appeared desirable to attempt the syntheses of esters which might possess similar hypotensive activities with lower emetic effects. White[11] prepared several germine esters by esterification of germine in pyridine with excess acylating agents, but none had appreciable activity. We now wish to report the preparation of a number of synthetic esters of high hypotensive activity by selective and stepwise esterification of germine. Most of the active natural esters possess two or three acid residues and in all esters at least one acid radical is α-branched and five-membered; it seemed logical, therefore, to prepare esters using acids of similar structure. In addition, in order to compare the extent of esterification and size of the acid residue with the activity of the ester, several series of mono-, di-, tri- and tetraesters were synthesized.

The D-(−)-methylethylacetic acid used for esterifications was isolated from the basic hydrolysate of an extract of *Veratrum viride* containing mixed esters of germine. The L-(+)-methylethylacetic acid was obtained by oxidation of the corresponding optically active alcohol, L-(−)-2-methylbutanol-1,[12] found in fusel oil.

The monoesters, III and IV, were prepared by treatment of germine in pyridine solution with one equivalent of the acid chloride followed by chromatography of the product on acid-washed alumina. The monoesters, I and II, were obtained as by-products in the preparation of the diisobutyrate (VI) and the didiethylacetate (VII) esters of germine. The acetonide monoisobutyrate (V) was obtained in addition to the acetonide diisobutyrate (XVIII) when germine acetonide was treated with excess isobutyryl chloride. The rotations of I, II, III and IV were all of the same order of magnitude suggesting that the same hydroxyl group of germine was esterified in each case. Although germine mono-D-(−)-methylethylacetate (III) is isomeric with protoveratridine,[10,13] a comparison of their physical properties and infrared spectra showed they were not identical. The diesters, VI through XV, were obtained as the major product when germine was treated with two equivalents of the corresponding acid chloride in pyridine. The diester, XVI, was obtained by treating germine mono-D-(−)-methylethylacetate with one equivalent of isobutyryl chloride in pyridine. The diesters, XVII and XVIII, were prepared from germine acetonide by acylation using excess acetic anhydride and isobutyryl chloride, respectively. Germine diacetate (XIX) was formed by decomposition of the acetonide, XVII, with 2,4-dinitrophenylhydrazine. No germine diisobutyrate could be isolated when this method of decomposition was applied to the acetonide diisobutyrate (XVIII). The rotational values of the aliphatic, synthetic diesters (with the exception of germine dipivalate) correspond closely to the values found for the naturally occurring diesters and this suggests strongly that direct esterification of germine introduces acid residues on the same hydroxyl groups found esterified in the natural products.[14] The high negative rotation observed for germine dipivalate would indicate that the large steric requirement of pivalyl chloride forces esterification of a different hydroxyl group. Different hydroxyl groups are also esterified in the diacetate XIX since it was prepared through the germine acetonide which apparently blocks at least one of the hydroxyl groups normally esterified first. In the case of germine difuroate (XV) the presence of unsaturation in the acid residues probably accounts for the large shift from the normal rotational value observed for aliphatic germine diesters.

The triesters, XX, XXI, XXII and XXIII, were prepared by treating the corresponding diesters, VI, VIII, IX and X, with one equivalent of acid anhydride in pyridine. Formation of these triesters results in a large negative shift in the rotation corresponding to values found for the natural triesters such as XXIX and XXX. This similarity in optical rotation as well as hypotensive activity

(1) A portion of this work was first described in a preliminary communication in *Chemistry and Industry*, 197 (1953).

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(3) E. D. Freis, J. R. Stanton and F. C. Moister, *J. Pharmacol. Expt. Therap.*, **98**, 166 (1950).

(4) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950).

(5) J. Fried, P. Numerof and N. H. Coy, *ibid.*, **74**, 3041 (1952).

(6) G. S. Meyers, W. L. Glen, P. Morozovitch, R. Barber and C. A. Grant, *ibid.*, **74**, 3198 (1952).

(7) M. W. Klohs, F. Keller, S. Koster and W. Malesh, *ibid.*, **74**, 1871 (1952).

(8) M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, *ibid.*, **74**, 4473 (1952).

(9) H. A. Nash and R. M. Brooker, *ibid.*, **75**, 1942 (1953).

(10) S. M. Kupchan and C. D. Deliwala, *ibid.*, **74**, 3202 (1952).

(11) H. L. White, *ibid.*, **73**, 492 (1951).

(12) We wish to thank Professor S. Winstein for his generous gift.

(13) W. Poethke, *Arch. Pharm.*, **275**, 571 (1937).

(14) This suggestion is further strengthened by the synthesis of the naturally occurring diester, germidine, from germine. A paper describing this work is now being prepared.

leaves little doubt that the same hydroxyl groups are esterified in both the natural and synthetic triesters.[15]

The tetraesters, XXV, XXVI, XVII and XXVIII, were obtained by acylation of the related diesters with excess of acid anhydride in pyridine.

All of the monoesters showed a low order of activity. The diesters which carried a branched α-methyl group, however, showed a high order of activity, the mixed ester, XVI, being the most active diester of germine synthesized. The cyclic diester, XIV, having a five-carbon acid residue also retained high activity. When the methyl branching occupies the β-carbon atom as in the diisovalerate (XII), isomeric with VIII and IX, the majority of activity is lost. In addition, little hypotensive activity was retained when the diester contained an α-ethyl branch, as illustrated by the didiethylacetate (VII). A straight chain arrangement of the carbon atoms in the diester, such as the di-*n*-butyrate (XI), also showed a marked decrease in activity compared with the isomeric diisobutyrate.

The most potent naturally occurring hypotensive agents are the triesters of germine. This was also found to be true in the synthetic esters. The triesters, XX and XXI, were highly active having roughly the same potency as the most active natural esters, germitrine (XXIX) and neogermitrine (XXX). In three particular series studied, the activities of the diester monoacetates (XX, XXI, XXII) were invariably larger than their diester precursors, and the relative potencies of the diesters (VI > VIII > IX) were retained in the corresponding triesters (XX > XXI > XXII). It is interesting that the synthetic esters containing the D-(−)-methylethylacetyl radical (found in naturally occurring esters) were always more potent than the corresponding esters containing the L-(+)-form of this acid.

Increasing the size of the third acid residue, exemplified by the series diisobutyrate acetate (XX), diisobutyrate propionate (XXIII) and triisobutyrate (XXIV),[11] served progressively to decrease the activity of the triester. This fact, together with the observation that only the four- or five-carbon acid residues are efficacious in producing a large hypotensive response, suggests that the approach of the germine ester molecule to a certain optimum dimension is an important factor in determining activity.

All the synthetic tetraesters were essentially impotent.

None of the synthetic esters showed a significantly more favorable emetic ratio than the natural ones.

The hypotensive evaluations were carried out according to the method of Maison and Stutzman.[16]

Acknowledgments.—We are indebted to Mr. M. A. Robinson and Mr. C. H. Stimmel for the determination of physical constants and to Dr. A. Elek for the microanalyses.

(15) Further support for this statement has been obtained by the synthesis of monoacetylneogermitrine from germine. This work will be described in a subsequent paper.

(16) G. L. Maison and J. W. Stutzman, *Arch. Int. Pharmacodyn.*, **85**, 357 (1951); *J. Pharm. Expt. Therap.*, **103**, 74 (1951).

Experimental[17]

D-(−)-Methylethylacetic Acid.—An extract of *Veratrum viride* (representing 80 kg. of root) containing mixed esters of germine was subjected to basic hydrolysis and after removal of the germine, the basic aqueous solution was steam distilled to remove volatile neutral material. The residue was then acidified with concentrated sulfuric acid and the solution distilled until the optical rotation of the distillate was 0°. The distillate was saturated with sodium chloride, extracted with ether, the combined extracts dried over magnesium sulfate and the ether removed by fractional distillation. The remaining oil was fractionally distilled and D-(−)-methylethylacetic acid was obtained as the major fraction (9.0 g.), b.p. 172–174°, $[\alpha]^{24}D$ −18.0° (pure liquid) (reported[18] $[\alpha]^{20}D$ −17.85°).

The acid chloride was prepared by reaction with thionyl chloride in the usual manner, b.p. 117°, $[\alpha]^{24}D$ −18° (pure liquid).

L-(+)-Methylethylacetic Acid.—Fourteen grams of potassium hydroxide and 117 g. of potassium permanganate were dissolved in 2 liters of water. To this solution was added 44 g. of L-(−)-2-methylbutanol over a period of one hour at 25°. The mixture was then stirred an additional hour, filtered to remove manganese dioxide and extracted with ether. The aqueous solution was treated with sodium sulfite to reduce any excess permanganate, acidified with hydrochloric acid and the methylethylacetic acid extracted with four 500-ml. portions of ether. The ether extracts were dried, concentrated by fractional distillation and the crude acid fractionated through a Claisen head at reduced pressure. L-(+)-Methylethylacetic acid (32.5 g., 64%) was collected at 90–94° at 23 mm., $[\alpha]^{25}D$ +19.2° (pure liquid).

The acid chloride was prepared by reaction with thionyl chloride at room temperature in the usual manner, b.p. 115°, $[\alpha]^{24}D$ +17° (pure liquid).

Germine Mono-L-(+)-methylethylacetate (IV).—To a cold (0°) solution of 2.13 g. of germine in 20 ml. of pyridine was added 0.60 g. of L-(+)-methylethylacetyl chloride. After standing at room temperature overnight, the solution was poured onto a slurry of ice and 10 ml. of 10% sodium carbonate. The oily mixture was extracted several times with chloroform, the combined extracts dried over magnesium sulfate and then taken to dryness *in vacuo*. The amorphous residue (2.69 g.) was dissolved in 15 ml. of benzene and chromatographed on 40 g. of Merck acid-washed alumina. Elution with 50% benzene–chloroform afforded 0.3 g. of germine di-L-(+)-methylethylacetate (IX). Further elution with 1 to 3% methanol–chloroform yielded 0.9 g. (36%) of the mono-L-(+)-methylethylacetate which crystallized from chloroform–ether in small colorless plates, m.p. 238–239°.

Germine Mono-D-(−)-methylethylacetate (III).—This ester was prepared in the same manner and yield as the mono-L-(+)-ester (IV). Recrystallization from chloroform–ether yielded colorless plates, m.p. 236–237°.

Germine Monoisobutyrate (I) and Germine Diisobutyrate (VI).—To a cold (0°) solution of 2.20 g. of germine in 25 ml. of dry pyridine was added 1.017 g. of isobutyryl chloride (or an equivalent amount of isobutyric anhydride). The solution was allowed to warm to room temperature and stand an additional 16 hours. The reaction mixture was poured onto crushed ice and 15 ml. of 10% sodium carbonate and the crude esters extracted from the basified solution with several portions of chloroform. The chloroform extracts were dried over magnesium sulfate, evaporated to dryness in vacuum and the amorphous residue chromatographed on 40 g. of Merck acid-washed alumina. Germine triisobutyrate (XXIV) (0.10 g.) was eluted with benzene, germine diisobutyrate (VI) (1.14 g., 41%) with 1% methanol–benzene and germine monoisobutyrate (0.05 g.) with methanol.

Germine triisobutyrate crystallized from benzene, m.p. 198–200° (reported[11] 197–201°). Germine diisobutyrate recrystallized from chloroform–ether in colorless prisms, m.p. 257–258°, and germine monoisobutyrate yielded colorless prisms, m.p. 248°, on recrystallization from benzene–petroleum ether.

Germine Monodiethylacetate (II) and Germine Didiethylacetate (VII).—These esters were prepared in the same manner as VI. The crude amorphous ester mixture was

(17) All melting points are corrected.

(18) O. Schutz and W. Marchwalk, *Ber.*, **29**, 52 (1896).

TABLE I

SYNTHETIC ESTERS OF GERMINE

	Germine ester	Formula	M.p., °C.	$[\alpha]^{24}D$[a]	[M]D	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Relative potency, % (germitrine) = 100%[b]
	Monoester									
I	Monoisobutyrate	$C_{31}H_{49}O_9N$	248	−21.1 − 7.0 (A)	−122	64.30	64.36	8.52	8.42	1
II	Monodiethylacetate	$C_{33}H_{53}O_9N$	213–215	−19.6	−122	65.21	65.12	8.79	8.98	<0.4
III	Mono-D-(−)-methylethylacetate	$C_{32}H_{51}O_9N$	236–238	−25.6	−154	64.73	65.09	8.66	8.67	3
IV	Mono-L-(+)-methylethylacetate	$C_{32}H_{51}O_9N$	238–239	−17.5	−107	64.73	64.36	8.66	8.70	0.3
V	Acetonide monoisobutyrate	$C_{31}H_{53}O_9N$	272–274	+57.5 (A)		65.86	65.98	8.62	8.87	0.09
	Diester									
VI	Diisobutyrate	$C_{35}H_{55}O_{10}N$	257–258	− 7.5 +31 (A)	− 49	64.70	64.79	8.53	8.48	27
VII	Didiethylacetate	$C_{39}H_{63}O_{10}N$	207–209	− 4.5	− 32	66.35	66.78	8.99	8.94	2
VIII	Di-D-(−)-methylethylacetate	$C_{37}H_{59}O_{10}N$	222–223	−11.8	− 81	65.55	65.56	8.77	8.39	16
IX	Di-L-(+)-methylethylacetate	$C_{37}H_{59}O_{10}N$	198	− 4.3	− 27	65.55	65.31	8.87	8.84	10
X	Dipropionate	$C_{33}H_{51}O_{10}N$	239–241	−15.4 +28.1 (A)	− 93	63.75	63.45	8.27	8.35	4
XI	Di-*n*-butyrate	$C_{35}H_{55}O_{10}N$	211–215	− 3.9	− 24	64.69	64.79	8.53	8.29	3
XII	Diisovalerate	$C_{37}H_{59}O_{10}N$	201–202	− 3.1	− 20	65.60	65.55	8.59	9.01	5
XIII	Dipivalate	$C_{37}H_{59}O_{10}N$	250–251	−39.2	−264	65.55	65.17	8.77	8.63	0
XIV	Dicyclobutanecarboxylate	$C_{37}H_{55}O_{10}N$	226–228	+ 2.1	+ 14	65.96	65.58	8.23	8.12	24
XV	Difuroate	$C_{37}H_{47}O_{12}N$	242–244	+82.2	+572	63.70	63.77	6.79	6.79	0.7
XVI	Mono-D-(−)-methylethylacetate monoisobutyrate	$C_{36}H_{57}O_{10}N$	252–253	− 9.7	− 66	65.15	65.32	8.66	8.38	31
XVII	Acetonide diacetate	$C_{34}H_{53}O_{10}N$	198	+31.6 (A)	...	64.32	64.13	8.06	7.80	<0.4
XVIII	Acetonide diisobutyrate	$C_{38}H_{61}O_{10}N$	190	+41.4 (A)	...	66.16	65.74	8.62	8.77	0.3
XIX	Diacetate	$C_{31}H_{47}O_{10}N$	210–213	+10 (A)	...	62.71	62.32	7.98	7.73	<4
	Triester									
XX	Diisobutyrate monoacetate	$C_{37}H_{57}O_{11}N$	249–251	−71	−491	64.24	63.99	8.31	8.33	77
XXI	Di-D-(−)-methylethylacetate monoacetate	$C_{39}H_{61}O_{11}N$	243–245	−60.3	−433	65.06	64.94	8.54	8.60	53
XXII	Di-L-(+)-methylethylacetate monoacetate	$C_{39}H_{61}O_{11}N$	228–230	−48.1	−332	65.06	65.03	8.54	8.65	21
XXIII	Diisobutyrate monopropionate	$C_{38}H_{59}O_{11}N$	240–241	−69.5	−494	64.66	64.56	8.43	8.76	23
XXIV	Triisobutyrate[11]	$C_{39}H_{61}O_{11}N$	198–200	−66	−475	65.07	64.82	8.54	8.26	4.5
	Tetraester									
XXV	Diisobutyrate diacetate	$C_{39}H_{59}O_{12}N$	252–253	−83	−609	63.83	63.76	8.10	8.38	1
XXVI	Diisobutyrate dipropionate	$C_{41}H_{63}O_{12}N$	249–250	−88	−671	64.58	64.98	8.33	8.34	0.3
XXVII	Di-D-(−)-methylethylacetate diacetate	$C_{41}H_{63}O_{12}N$	257–258	−80	−538	64.58	64.50	8.33	8.68	0.5
XXVIII	Di-L-(+)-methylethylacetate diacetate	$C_{41}H_{63}O_{12}N$	252–253	−70	−470	64.58	64.49	8.33	8.61	<1
	Naturally occurring ester									
XXIX	Germitrine		214–218	−69						100
XXX	Neogermitrine		234–235	−79						64
XXXI	Germidine		231–233	−11						21

[a] All rotations in pyridine except as indicated: (A) = 95% ethanol. [b] Determined by the method of G. L. Maison and J. W. Stutzman.[16]

chromatographed on Merck acid-washed alumina. The diester was eluted from the column with benzene and the monoester with 25% chloroform–benzene. Recrystallization from acetone–water yielded white prisms of diester, m.p. 207–209°. The monoester crystallized from chloroform–ether in colorless prisms, m.p. 213–215°.

Germine Di-D-(−)-methylethylacetate (VIII).—Two grams of D-(−)-methylethylacetyl chloride was added to a cold (0°) solution of dry pyridine containing 3.83 g. of dissolved germine. The reaction mixture (protected by a drying tube) was allowed to stand at room temperature for 16 hours, then poured onto a mixture of ice and 10 ml. of 10%

sodium carbonate. The crude ester was extracted with chloroform, the extract dried over anhydrous magnesium sulfate and the chloroform removed *in vacuo*. The amorphous residue which remained was chromatographed on 110 g. of Merck acid-washed alumina. Elution with 1% methanol–benzene gave 2.2 g. (43%) of diester which crystallized from chloroform–ether in colorless prisms, m.p. 222–223°.

Germine Di-L-(+)-methylethylacetate (IX).—This ester was prepared by the method used for compound VIII except that the ester was eluted with $^1/_2$–1% methanol–benzene (40% yield). Recrystallization from chloroform–ether gave colorless prisms, m.p. 198°.

Germine Dipropionate (X).—This compound was prepared by the method used to obtain the diisobutyrate (VI). The product was chromatographed on Merck acid-washed alumina. The ester was eluted with 50% chloroform–benzene (36%) and on recrystallization from chloroform–ether yielded colorless prisms, m.p. 239–241°.

Germine Di-*n*-butyrate (XI).—This ester was prepared in the same manner as compound VI. The crude ester was chromatographed on Merck acid-washed alumina, eluted from the column with 50% benzene–chloroform (6%) and recrystallized from chloroform–ether in colorless prisms, m.p. 211–215°.

Germine Diisovalerate (XII).—The ester was prepared by the method used for compound VI. The crude ester was chromatographed on Merck acid-washed alumina. Elution with chloroform and 1% methanol–chloroform yielded the diester (49%) which crystallized from chloroform–ether in colorless prisms, m.p. 201–202°.

Germine Dipivalate (XIII).—One gram of trimethylacetyl chloride was added to 2.00 g. of germine dissolved in 30 ml. of pyridine at 0°. After 16 hours the reaction mixture was worked up in the same manner as VI. The crude ester was chromatographed on 50 g. of Merck acid-washed alumina. Elution with 25% chloroform–benzene gave 0.10 g. of dipivalate ester which was recrystallized from chloroform–ether in colorless prisms, m.p. 250–251°.

Germine Dicyclobutanecarboxylate (XIV).—This ester was prepared and worked up in the same manner as VI. It was eluted from the column with 20 to 75% chloroform–benzene (30% yield). Crystallization from chloroform–ether yielded a colorless crystalline solid, m.p. 223–227°.

Germine Difuroate (XV).—The difuroate was prepared in the same manner as VI. The diester was eluted from a column of Merck acid-washed alumina with chloroform and recrystallized from chloroform–ether as colorless prisms (37%), m.p. 242–244°.

Germine Mono-D-(−)-methylethylacetate Monoisobutyrate (XVI).—To a solution of 0.954 g. of germine mono-D-(−)-methylethylacetate (III) in 10 ml. of pyridine was added 0.195 g. of isobutyryl chloride. After standing overnight the reaction mixture was worked up in the same manner as VI. The crude ester was chromatographed on 25 g. of Merck acid-washed alumina and eluted from the column with 50% chloroform–benzene, 0.25 g. (24%). It was crystallized from acetone–water as colorless needles, m.p. 252–253°.

Germine Monoacetonide Diacetate (XVII).—A mixture of 1.00 g. of germine acetonide,[19] 30 ml. of pyridine and 25 ml. of acetic anhydride was allowed to stand at room temperature overnight. The excess pyridine and acetic anhydride were removed under vacuum leaving a brown solid which after three crystallizations from benzene–petroleum ether yielded the acetonide diacetate (0.80 g.) as a colorless solid, m.p. 198°.

Anal. Calcd. for $C_{34}H_{53}O_{10}N$: 2 CH_3CO, 13.5. Found: CH_3CO, 13.1.

Germine Monoacetonide Monoisobutyrate (V) and Germine Monoacetonide Diisobutyrate (XVIII).—A mixture of 1.00 g. of germine monoacetonide, 20 ml. of pyridine and 0.75 ml. of isobutyryl chloride was allowed to stand overnight at room temperature. The reaction mixture was concentrated *in vacuo* to an oil, made basic with 15 ml. of 10% sodium carbonate and then extracted with chloroform. The chloroform extract was dried, evaporated to dryness *in vacuo* and the crude residue (1.2 g.) chromatographed on 30 g. of acid-washed alumina. The column was eluted with benzene, $^1/_2$% chloroform–benzene and 1% chloroform–benzene. These combined fractions yielded the monoacetonide diisobutyrate. With 10% chloroform–benzene, the monoacetonide monoisobutyrate was obtained. The acetonide diisobutyrate was crystallized from chloroform–petroleum ether as colorless prisms, m.p. 190°. The acetonide monoisobutyrate crystallized from benzene–petroleum ether in colorless prisms, m.p. 272–274°.

Germine Diacetate (XIX).—A solution of 300 mg. of germine monoacetonide diacetate (XVII) in a minimum amount of ethanol was added to a solution of 89.6 mg. of 2,4-dinitrophenylhydrazine in 0.5 ml. of concentrated sulfuric acid and 0.25 ml. of water. After standing 1.5 hours, acetone 2,4-dinitrophenylhydrazone had separated from the solution. The solution was diluted with water, filtered and extracted with chloroform. The chloroform extracts (dried over magnesium sulfate) were evaporated under vacuum leaving a residue (200 mg.) which was crystallized from chloroform–petroleum ether, colorless prisms, m.p. 210–213°.

Anal. Calcd. for $C_{31}H_{47}O_{10}N$: 2 CH_3CO, 14.5. Found: CH_3CO, 13.0.

Germine Diisobutyrate Monoacetate (XX).—To a solution of 1.18 g. of germine diisobutyrate (VI) in 5 ml. of pyridine at 0° was added 0.218 g. of acetic anhydride. After standing overnight the excess pyridine was removed under vacuum and the resulting white powder dissolved in 25 ml. of cold chloroform. This solution was saturated with ammonia gas, cooled and filtered to remove any ammonium acetate present. The chloroform was removed under vacuum and the residue was recrystallized from acetone–water, colorless plates, m.p. 228–230° (1.0 g., 80%).

Germine Di-D-(−)-methylethylacetate Monoacetate (XXI).—This ester was prepared by the same method as XX. The triester was recrystallized from acetone–water, colorless plates (65%), m.p. 234–235°.

Germine Di-L-(+)-methylethylacetate Monoacetate (XXII).—This compound was prepared by the same method as XX. It was recrystallized from acetone–water, colorless plates (71%), m.p. 228–230°.

Germine Diisobutyrate Monopropionate (XXIII).—A solution of 2.50 g. of germine diisobutyrate (VI) in 15 ml. of pyridine was cooled to 0° and treated with 0.50 ml. of propionic anhydride. After the reaction mixture stood overnight at room temperature, it was worked up in the same manner as XX except that the product was chromatographed on 50 g. of acid-washed alumina. The triester was eluted with 10–25% chloroform–benzene (0.5 g., 18.5%) and recrystallized twice from acetone–water (0.10 g.), colorless prisms, m.p. 240–241°.

Germine Diisobutyrate Diacetate (XXV).—A solution containing 1.50 g. of germine diisobutyrate (VI), 5 ml. of pyridine and 5 ml. of acetic anhydride was allowed to stand at room temperature overnight. The excess solvent was removed *in vacuo* and the residue dissolved in chloroform saturated with ammonia gas. The solution was filtered and evaporated to dryness leaving an amorphous solid which crystallized from acetone in colorless plates, m.p. 253–254° (1.50 g., 88%).

Germine Diisobutyrate Dipropionate (XXVI).—This ester was prepared from VI in the same manner as XXV. It was recrystallized from acetone–water in colorless plates (70%), m.p. 249–250°.

Germine Di-D-(−)-methylethylacetate Diacetate (XXVII).—This compound was prepared from VIII in the same manner as XXV. Crystallization from acetone–water gave colorless needles (81%), m.p. 257–258°.

Germine Di-L-(+)-methylethylacetate Diacetate (XXVIII).—This ester was prepared from IX by the method used for XXV. Recrystallization from acetone–water gave colorless needles (85%), m.p. 252–253°.

(19) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **148**, 57 (1943).

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