N-METHYL ANALOGUES OF SOME PEPTIDE JUVENOIDS

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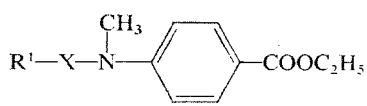
The synthesis and biological properties of derivatives of some amino acids and N-methyl amino acids containing the ethyl 4-aminobenzoate and ethyl 4-methylaminobenzoate have been described. These substances were substituted at the N-terminal by *p*-toluenesulfonyl, tert-butyloxycarbonyl, pivaloyl or chloroacyl groups. Substitution of one or both amide bonds of previously described peptide juvenile hormones by a methyl group resulted in a decrease in juvenile hormone activity by 1—2 orders of ten.

In the course of studies of the biological and physical properties of derivatives of N-methyl amino acids it was of interest to observe the effect of substitution of a methyl group on the nitrogen of the amide bond on juvenile hormone activity of highly active analogues (juvenoids) prepared previously[1-3]. A number of N-methyl analogues of these juvenoids were prepared: derivatives of L-alanine, N-methyl-L-alanine, L-valine, N-methyl-L-valine and sarcosine, containing the ethyl 4-aminobenzoate or ethyl 4-methylaminobenzoate. These compounds were blocked at the N-terminal, either with a tert-butyloxycarbonyl or *p*-toluenesulfonyl group or a residue of pivalic, trichloroacetic, 2,2'-dichloropropionic or α-chloroisobutyric acids.

Preparation of derivatives of N-methyl amino acids presents a complex synthetic problem. Acylation of N-methyl amino acids and their derivatives, as opposed to the amino acids themselves, gives lower yields because of the steric effect of the methyl group preventing access of further substituent to the nitrogen atom. This effect is stronger than the positive induction effect of the methyl group itself. The synthesis is also complicated by the difficulty of crystallizing derivatives of N-methyl amino acids, so that pure substances must be isolated by chromatography.

Even with reactions of acylamino acids with ethyl 4-aminobenzoate, described elsewhere[1-3], there were low yields because of the lower reactivity of an amino group bound to an aromatic nucleus, even more so when substituted in position 4 with an electronegative ester group. For this reason, in the present work for preparation of substances *I* and *II* by condensation of the ethyl 4-methylaminobenzoate with benzyloxycarbonyl-L-valine[4] or *p*-toluenesulfonyl-N-methyl-L-valine by means

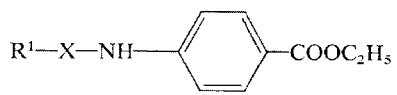
of PCl_3 in pyridine[5], it was necessary to prolong the usual 3-hour reaction time by a factor of 4. *p*-Toluenesulfonyl-N-methyl-L-valine was prepared from tert-butyl-ester of *p*-toluenesulfonyl-L-valine, obtained from the reaction of *p*-toluenesulfonyl--L-valine[6] with isobutylene, methylation with dimethylsulfate in NaOH and subsequent hydrolysis with trifluoroacetic acid. In order to make sure of the optical purity of *p*-toluenesulfonyl-N-methyl-L-valine prepared by the above technique of methylation, tert-butyl ester of *p*-toluenesulfonyl-N-methyl-L-valine was hydrolyzed by 30 min of boiling with hydrobromic acid in the presence of phenol[7] and from the resulting hydrobromide N-methyl-L-valine was freed up on the ion exchanger Zerolite 225; optical rotation was in agreement with the published data[8,9]. With substance *I* the benzyloxycarbonyl protecting group was finally removed by using 36% HBr in acetic acid and base was freed from the hydrobromide by a chloroformic solution of ammonia, and on reaction with trichloroacetic acid, 2,2′-dichloropropionic acid and α-chloroisobutyric acid by the action of phosphorus oxychloride in tetrahydrofurane and pyridine substances *III*–*V* yielded after going through a salt stage resulting before the condensation reaction by mixing the components in ether[10]. The same methods were used to prepare substances *VI* and *VII*, condensation of 2,2′-dichloropropionic and α-chloroisobutyric acids with the ethyl N-methyl--L-valyl-4-methylaminobenzoate. The latter substance was obtained by removal of the protecting *p*-toluenesulfonyl group in compound *II*, heating with 36% HBr



I, $R^1 = C_6H_5CH_2OCO$, X = Val
II, $R^1 = CH_3C_6H_4SO_2$, X = MeVal
III, $R^1 = Cl_3CCO$, X = Val
IV, $R^1 = CH_3Cl_2CCO$, X = Val
V, $R^1 = (CH_3)_2ClCCO$, X = Val
VI, $R^1 = CH_3Cl_2CCO$, X = MeVal
VII, $R^1 = (CH_3)_2ClCCO$, X = MeVal
VIII, $R^1 = (CH_3)_3CCO$, X = Val
IX, $R^1 = (CH_3)_3CCO$, X = Ala

in acetic acid in the presence of phenol[11] and freeing up base from the hydrobromide by ammonia in chloroform. Substances *VIII* and *IX* were obtained by condensation of pivaloyl-L-valine or pivaloyl-L-alanine with the ethyl 4-methylaminobenzoate using dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole[12], but it was necessary to use three times the amount of base and twice the reaction time. Substance *X* was prepared by reaction of *p*-toluenesulfonyl-N-methyl-L-valine with the ethyl 4-aminobenzoate by the action of PCl_3 in pyridine[5]. Using detosylation described for substance *II* the ethyl N-methyl-L-valyl-4-aminobenzoate was prepared

and by acylation with pivaloyl chloride in the presence of N-ethylpiperidine in ether compound *XI* was obtained. The preparation of substance *XII* was started with N-methyl-L-alanine, prepared in the following manner: *p*-toluenesulfonyl-L-alanine[13] was transformed into the isopropyl ester using the method of Brenner[14] and was then methylated with dimethyl sulfate in NaOH, and from the resulting isopropyl ester of *p*-toluenesulfonyl-N-methyl-L-alanine, N-methyl-L-alanine was prepared in the same manner as N-methyl-L-valine from tert-butylester of *p*-toluenesulfonyl--N-methyl-L-valine. Starting from N-methyl-L-alanine, the optical rotation of which was in agreement with the published data[9], the methyl ester of N-methyl-L-alanine--hydrochloride was prepared and base was freed by ammonia in chloroform. After acylation of the free ester with pivaloyl chloride in ether in the presence of N-ethylpiperidine, the resultant methyl ester of pivaloyl-N-methyl-L-alanine was saponified. Pivaloyl-N-methyl-L-alanine was condensed with the ethyl 4-aminobenzoate by the action of PCl_3 in pyridine[5] to yield substance *XII*.



X, $R^1 = CH_3C_6H_4SO_2$, X = MeVal
XI, $R^1 = (CH_3)_3CCO$, X = MeVal
XII, $R^1 = (CH_3)_3CCO$, X = MeAla
XIII, $R^1 = (CH_3)_3COCO$, X = Sar-Ala
XIV, $R^1 = H$, X = Sar-Ala

Poduška and coworkers[2] previously reported that one of the conditions for juvenile hormone activity of peptide juvenoids is an optically active central amino acid in the L-configuration. We have also observed a marked dependence of activity on the length of the molecular chain[1,3]. These facts were taken into account in the preparation of substances *XIII* and *XIV*, where we started from the ethyl tert-butyloxycarbonyl-L-alanyl-4-aminobenzoate[2], from which the N-protecting group was removed and free base obtained as in the preparation of substance *I*. The resulting ethyl L-alanyl-4-aminobenzoate was then condensed with tert-butyloxycarbonyl sarcosine[15] using dicyclohexylcarbodiimide in chloroform to yield substance *XIII*. By removal of the tert-butyloxycarbonyl group by a procedure described in substance *I*, ethyl sarcosyl-L-alanyl-4-aminobenzoate (*XIV*) was obtained.

All these substances were tested for juvenile hormone activity on freshly hatched larvae of the last instar of the bug *Pyrrhocoris apterus* and *Dysdercus cingulatus* (*Pyrrhocoridae*), *Graphosoma italicum* (*Pentatomidae*) and in freshly hatched larvae of *Tenebrio molitor* (*Tenebrionidae*). Compounds showing activity were effective only on bugs of the family *Pyrrhocoridae*, just as substances described previously, and these are presented in Table II.

TABLE I

N-Methyl Analogues of Peptide Juvenile Hormones *I*—*XIII*

Substance (yield, %) Method	Acyl component Amino component	M.p., °C solvent	Formula (m.w.)	Calculated/Found % C	% H	% N	% Cl	$[\alpha]_D^{\circ}$ (*c*)
I (37)	Z-L-Val[a]	syrup[b]	$C_{23}H_{28}N_2O_5$	66·97	6·84	6·79	—	—
A	MePABE	—	(412·5)	66·96	6·83	6·75	—	—
II (28)	Tos-L-MeVal	115—117	$C_{23}H_{30}N_2O_5S$	61·86	6·77	6·27	7·18[c]	—
A	MePABE	[d]	(446·6)	62·24	6·78	6·23	7·35	—
III (34)	Cl_3CCOOH	120—122	$C_{17}H_{21}Cl_3N_2O_4$	48·19	5·00	6·61	25·10	+116·2
B	L-Val-MePABE	[e]	(423·7)	48·25	5·15	6·35	24·92	(0·15)
IV (32)	CH_3Cl_2CCOOH	102—104	$C_{18}H_{24}Cl_2N_2O_4$	53·61	6·00	6·95	17·58	+144·1
B	L-Val-MePABE	[e]	(403·3)	54·03	6·02	6·68	17·46	(0·19)
V (40)	$(CH_3)_2ClCCOOH$	74—76	$C_{19}H_{27}ClN_2O_4$	59·60	7·11	7·34	9·26	+169·2
B	L-Val-MePABE	[e]	(382·9)	59·30	7·02	7·48	9·11	(0·12)
VI (24)	CH_3Cl_2CCOOH	[f]	$C_{19}H_{26}Cl_2N_2O_4$	54·68	6·28	6·71	16·99	+ 2·7
B	L-MeVal-MePABE	—	(417·4)	54·49	6·14	6·95	17·08	(0·55)
VII (21)	$(CH_3)_2ClCCOOH$	[f]	$C_{20}H_{29}ClN_2O_4$	60·05	7·38	7·07	8·93	— 51·9
B	L-MeVal-MePABE	—	(396·9)	59·82	7·35	7·14	8·59	(0·64)
VIII (52)	Piv-L-Val	107—108	$C_{20}H_{30}N_2O_4$	66·27	8·37	7·73	—	+ 1·4
C	MePABE	[d]	(362·5)	66·33	8·39	7·38	—	(0·64)
IX (42)	Piv-L-Ala	[g]	$C_{18}H_{26}N_2O_4$	64·65	7·84	8·38	—	+ 1·2
C	MePABE	—	(334·4)	64·58	7·95	8·03	—	(0·52)
X (37)	Tos-L-MeVal	[f]	$C_{22}H_{28}N_2O_5S$	61·09	6·53	6·48	7·41[c]	+ 76·2
A	PABE	—	(432·5)	61·49	6·77	6·35	7·41	(0·51)
XI (88)	PivCl	[g]	$C_{20}H_{30}N_2O_4$	66·27	8·34	7·73	—	— 89·4
D	L-MeVal-PABE	—	(362·5)	66·04	8·45	7·91	—	(0·19)
XII (33)	Piv-L-MeAla	[g]	$C_{18}H_{26}N_2O_4$	64·65	7·84	8·38	—	+ 1·4
A	PABE		(334·4)	64·52	7·98	8·15	—	(0·53)
XIII (66)	BOC-Sar	[f]	$C_{20}H_{29}N_3O_6$	58·95	7·17	10·31	—	— 36·4
C	L-Ala-PABE	—	(407·5)	59·12	7·04	10·45	—	(0·52)

[a] Abbreviations are according to IUPAC-IUB rules[16]; PABE ethyl 4-amino benzoate; MePABE ethyl 4-methylamino benzoate; Piv pivaloyl; [b] chromatography in the following systems: *I* (TLC R_F 0·34) 60% ether–light petroleum, *II* 30% ethyl acetate–light petroleum. *III*, *VIII*, *XII* 10% ether–benzene, *IV*, *XI* 20% ether–benzene, *V* 40% benzene–ether, *VI* (TLC R_F 0·43) 10% ether–light petroleum, *VII* 20% ether–light petroleum, *IX* (TLC R_F 0·29) 50% ether–benzene, *X* 40% ether–light petroleum; [c] % S; [d] ether–light petroleum; [e] aqueous ethanol; [f] foam; [g] syrup.

EXPERIMENTAL

Melting points were determined on a Kofler block. Samples for elemental analysis were dried for several hours over phosphorus pentoxide at room temperature and 1 Torr. Evaporation was carried out on a rotating evaporator (water pump, bath temperature about 35°C). Mixtures containing dimethylformamide were evaporated at 1 Torr. Optical rotations of substances in dimethylformamide were determined on a Perkin Elmer 141 apparatus at 23°C. Electrophoresis was carried out after removal of the corresponding protecting groups, on paper Whatman 3 MM for 45 min at a potential drop of 20 V/cm, in 1M acetic acid (pH 2·4) and pyridine–acetate (pH 5·7) buffer with ninhydrin detection. Mass spectrograms were measured on the MS 902 AEI apparatus. Thin layer chromatography (TLC) was carried out on fluorescent silica gel of particle size up to 30 microns, column chromatography was carried out on silica gel of particle size 30—60 microns. Both materials were delivered by the Service Laboratories of this Institute.

TABLE II

Juvenile Hormone Activity of Substances and Their Corresponding Unmethylated Forms on the Bug *Dysdercus cingulatus*

Substance[a]	Activity[b]
Cl_3CCO-L-Val-PABE	0·009
Cl_3CCO-L-Val-MePABE (*III*)	1·0
CH_3Cl_2CCO-L-Val-PABE	0·005
CH_3Cl_2CCO-L-Val-MePABE (*IV*)	0·008
CH_3Cl_2CCO-L-MeVal-MePABE (*VI*)	1·0
$(CH_3)_2ClCCO$-L-Val-PABE	0·002
$(CH_3)_2ClCCO$-L-Val-MePABE (*V*)	0·05
$(CH_3)_2ClCCO$-L-MeVal-MePABE (*VII*)	10·0
Piv-L-Val-PABE	0·04
Piv-L-Val-MePABE (*VIII*)	8·0
Piv-L-MeVal-PABE (*XI*)	0·1
Piv-L-Ala-PABE	0·04
Piv-L-Ala-MePABE (*IX*)	0·07
Piv-L-MeAla-PABE (*XII*)	5·0

[a] For abbreviations see Table I; [b] substances were applied topically in 1 μl acetone solution. Activity was evaluated on the basis of inhibition of metamorphosis of the external morphological characters of the insect and expressed in units of activity corresponding to nanograms of substances which produced a 50% morphological effect under the given conditions. Only those substances are presented which have an observable effect.

Preparation of Compounds *I*—*XIII* (Table I)

Method A (ref.[5]): To a solution of the amino component (10 mM) in pyridine (25 ml) PCl_3 (0·33 ml with substances *I* and *II*, 0·50 ml with substances *X* and *XII*) was added at −20°C, then after 30 min at −20°C and after 30 min at room temperature the acyl component (10 mM) was added. The mixture was boiled under a reflux condensor for 12 h (substances *I* and *II*) or 3 h (substances *X* and *XII*) and after cooling and filtration with active charcoal the pyridine was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the solution was shaken up consecutively with 1M-HCl, water, 5% $NaHCO_3$ and then with water. After drying with anhydrous sodium sulfate the ethyl acetate was evaporated and the remaining syrup was chromatographed on silica gel.

Method B (ref.[10]): To a solution of the amino component (10 mM) in ether (25 ml) a solution of the chloroacid (10 mM) in ether (5 ml) was dropwise added. After evaporating the solvents, the residue was dissolved in tetrahydrofurane (30 ml) and phosphorus oxychloride (1 ml) was added. After cooling to −15°C, pyridine (2·4 ml) was added under stirring and the temperature was allowed to rise gradually to room temperature, the mixture being stirred for a further hour (with substances *VI* and *VII* for 16 h). Tetrahydrofurane was then distilled off and the remaining oil was extracted with ethyl acetate (3 times 50 ml). After washing the pooled extracts in 5% $NaHCO_3$, 1M-HCl and water and drying with anhydrous sodium sulphate the ethyl acetate was distilled off, and the remaining syrup was chromatographed on silica gel.

Method C (ref.[12]): 1-Hydroxybenzotriazole (11 mM) and dicyclohexylcarbodiimide (11 mM) were added with stirring at −7°C to a solution of the acyl component (10 mM) in dimethylformamide (with substance *XIII* the condensation was carried out in chloroform). After 30 min the amino component (30 mM) was added to the mixture at the same temperature (only 10 mM with substance *XIII*) and the mixture was maintained at 5°C for 72 hours. The dicyclohexylurea which had precipitated was then filtered off and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the solution was washed with 1M-HCl (substance *XIII* with 20% citric acid), water, 5% $NaHCO_3$, and then water, and after drying the solution with anhydrous sodium sulphate the ethyl acetate was evaporated under reduced pressure and the remaining syrup was chromatographed.

Method D: To a solution of the amino component (10 mM) in ether (20 ml) N-ethylpiperidine (1·37 ml) was added and pivaloyl chloride (10 mM) was added dropwise with stirring at 0°C. The mixture was left for 10 min at 5°C, the hydrochloride of N-ethylpiperidine was filtered off and washed with ether. After dilution with ethyl acetate the filtrate was washed with 1M-HCl, 5% $NaHCO_3$ and water and dried with anhydrous sodium sulphate. After distilling off the solvents an oily remnant was obtained, which was chromatographed on silica gel.

Ethyl Sarcosyl-L-alanyl-4-aminobenzoate (*XIV*)

A mixture of ethyl tert-butyloxycarbonylsarcosyl-L-alanyl-4-aminobenzoate (1·6 g) and 36% HBr in acetic acid (2 ml) at room temperature was diluted over 10 min with ether and after decanting the solvents the solid residue was 4 times extracted with ether. The remnant, after filtration and washing with ether, was suspensed in a chloroformic solution of ammonia and the mixture was shaken for 30 min at 0°C. Ammonium bromide was then filtered off, washed on the filter with chloroform and after distilling off the solvent, the remaining syrup was crystallized from a mixture of ether and light petroleum. After filtration there was a yield of 1·2 g of m.p. 93—97°C. Recrystallization from ethyl acetate–light petroleum gave a yield of 1·1 g (92%) m.p. 96—98°C, $[\alpha]_D^{23}$ −11·6 (*c* 0·38). For $C_{15}H_{21}N_3O_4$ (307·4) calculated: 58·61% C, 6·88% H, 13·64% N; found: 58·74% C, 7·01% H, 13·59% N.

Tert-Butyl Ester of *p*-Toluenesulfonyl-L-valine

p-Toluenesulfonyl-L-valine (27·1 g) was shaken in a pressure flask with isobutylene (270 ml) in dichloromethane (360 ml) in the presence of H_2SO_4 (1·5 ml) for 12 h and after evaporation of the solvents the light petroleum was added to the remainder, filtered, and it yielded 32·5 g, m.p. 153—156°C. Recrystallization from a mixture of ether and light petroleum gave a yield of 30·2 g (92%), m.p. 155—157°C, $[\alpha]_D^{23}$ —26·3 (*c* 0·46). For $C_{16}H_{25}NO_4S$ (327·5) calculated: 58·68% C, 7·68% H, 4·28% N, 9·79% S; found: 58·62% C, 7·73% H, 4·08% N, 10·02% S.

Tert-Butyl Ester of *p*-Toluenesulfonyl-N-methyl-L-valine

To a solution of tert-butyl ester of *p*-toluenesulfonyl-L-valine (32·7 g) in 4M-NaOH (50 ml) in the presence of saponate (2 ml) and after cooling with ice dimethyl sulfate (37·6 g) was added with stirring and then over 4 h 4M-NaOH (25 ml) in 5 aliquots was added. The mixture was then washed with ethyl acetate (3 times) and the pooled ethyl acetate extracts were washed with 4M--NaOH and water. After evaporation of the solvent there was a yield of 34·1 g with m.p. 60—62°C, after recrystallization from light petroleum 33·1 g (97%), m.p. 62—63°C, $[\alpha]_D^{23}$ —33·1° (*c* 0·54). For $C_{17}H_{27}NO_4S$ (341·5) calculated: 59·79% C, 7·97% N, 4·10% N, 9·39% S; found: 59·62% C, 8·07% H, 3·99% N, 9·71% S.

p-Toluenesulfonyl-N-methyl-L-valine Dicyclohexylammonium Salt

Tert-butyl ester of *p*-toluenesulfonyl-N-methyl-L-valine (34·2 g) was dissolved in trifluoroacetic acid (37·2 ml) at room temperature and after 2 h the mixture was evaporated. The remaining oil was evaporated 3 times with water and then with benzene and the final remnant was dissolved in ether (200 ml) and at 0°C dicyclohexylamine (19·6 ml) was added and the mixture was left at 5°C. After sucking off the supernatant and washing the product with ether there was a yield of 45·3 g of salt with m.p. 158—163°C. Recrystallization from the mixture of ethanol and ether gave a yield of 43·3 g (93·5%), m.p. 161—164°C, $[\alpha]_D^{23}$ —18·6°, (*c* 0·47). For $C_{25}H_{42}N_2O_4S$ (466·7) calculated: 64·37% C, 9·07% H, 6·00% N, 6·87% S; found: 64·22% C, 9·12% H, 6·23% N, 6·81% S.

Isopropyl Ester of *p*-Toluenesulfonyl-L-alanine

Esterification according to Brenner was used with *p*-toluenesulfonyl-L-alanine (24·3 g) to prepare 27·1 g of the substance, m.p. 82—85°C, recrystallized from ether and light petroleum. The final product was 25·2 g (88%) m.p. 85—86°C, $[\alpha]_D^{23}$ —30·8° (*c* 0·47). For $C_{13}H_{19}NO_4S$ (285·4) calculated: 54·71% C, 6·71% H, 4·91% N, 11·23% S; found: 54·48% C, 6·68% H, 4·75% N, 11·28% S.

N-Methyl-L-alanine

Using the same methylation approach as with tert-butyl ester of *p*-toluenesulfonyl-N-methyl-L-valine the isopropyl ester of *p*-toluenesulfonyl-L-alanine (28·5 g) was used to prepare the isopropyl ester of *p*-toluenesulfonyl-N-methyl-L-alanine (29·2 g) as an oil. This N-methyl derivative (29·9 g) was boiled for 30 min with hydrobromic acid (180 ml) and phenol (29·4 g). After evaporation and repeated evaporation with water (4 times) the aqueous solution was put onto a column with Zerolit 225 and after washing out the bromide ions with water the N-methyl-L-alanine was eluted with 10% pyridine. After evaporating the pyridine the remainder was washed with ethanol and

after drying there was a yield of 7·6 g, m.p. 257—262°C. After recrystallization from aqueous ethanol the yield was 7·33 g (71%), m.p. 260—262°C, $[\alpha]_D^{23}$ +11·2° (*c* 0·56, 6M-HCl). For $C_4H_9.NO_2$ (103·1) calculated: 46·60% C, 8·79% H, 13·59% N; found: 46·57% C, 8·85% H, 13·59% N.

N-Methyl-L-valine

This substance was prepared from tert-butyl ester of *p*-toluenesulfonyl-N-methyl-L-valine (3·4 g) using the same method as for N-methyl-L-alanine from the isopropyl ester of *p*-toluenesulfonyl-N-methyl-L-alanine, with a yield of 0·96 g (73%), m.p. 298—300°C, $[\alpha]_D^{23}$ +31·1° (*c* 0·52, 6M-HCl). For $C_6H_{13}NO_2$ (131·2) calculated: 54·93% C, 9·99% H, 10·68% N; found: 54·83% C, 10·05% H, 10·81% N.

Pivaloyl-N-methyl-L-alanine

N-Methyl-L-alanine (5·2 g) was esterified with methanol and HCl and base freed up with the action of ammonia in chloroform. N-Ethylpiperidine (4·2 ml) was added to a solution of the methyl ester of N-methyl-L-alanine (3·5 g) in ether (50 ml) and further added dropwise with mixing pivaloyl chloride (3·6 ml). The hydrochloride of N-ethylpiperidine which precipitated was filtered and the filtrate was washed with 1M-HCl, 5% $NaHCO_3$ and water. After drying the solution with sodium sulphate the ether was distilled off, the oily methyl ester of pivaloyl-N-methyl-L-alanine was saponified after dissolving in acetone (15·9 ml). Saponification was carried out by means of 1M-NaOH (10·6 ml) added for more than 1 hour. After concentrating the solution to about 15 ml and washing it with ether, it was acidified with 1M-HCl and the product was extracted into ether. Dicyclohexylamine was added to the ether solution (4·8 ml) and the product which precipitated was filtered and washed with ether. The yield was 6·25 g of the dicyclohexylammonium salt of pivaloyl-N-methyl-L-alanine, m.p. 151—153°C. After recrystallization from ethyl acetate and light petroleum the yield was 4·9 g, m.p. 152—154°C, $[\alpha]_D^{23}$ −16·5° (*c* 0·54) For $C_{21}H_{40}N_2O_3.H_2O$ (370·6) calculated: 68·06% C, 11·42% H, 7·56% N; found: 68·21% C, 11·02% H, 7·63% N.

Pivaloyl-N-methyl-L-alanine was freed by mixing the dicyclohexylammonium salt in 50% ethanol with Dowex 50 W for 30 min, the Dowex was then filtered off, washed with 50% ethanol, the solvents were distilled off and the remainder was evaporated 3 times with benzene. The yield was 2·5 g of m.p. 115—119°C, recrystallization from ether and light petroleum gave a yield of 2·3 g (25%, calculated on N-methyl-L-alanine), m.p. 117—120°C. For $C_9H_{17}NO_3$ (187·2) calculated: 57·75% C, 9·15% H, 7·48% N; found: 57·61% C, 8·95% H, 7·81% N.

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