A second alkaloid extracted by chloroform at pH 5, crystallized directly and after recrystallization from hexane had m.p. 274–275°, $[\alpha]_D^{25}$ +108° (*c*, 1.01 in pyridine) I_2 then $Ce(SO_4)_2$ colour blue-green. High resolution mass spectrometry[4] established the molecular formula $C_{42}H_{54}N_4O_6$ (found, *M* 710.40631; calc., *M* 710.40431). IR-absorption at 2.92 and 6.11 μ indicated the presence of hydroxyl and strongly hydrogen-bonded amide groups as present in I. The UV-spectrum, λ_{max}^{EtOH} 236 and 297 nm (ε 19,500, 11,500), λ_{min}^{EtOH} 262 nm (ε 3550), $\lambda_{max}^{EtOH-NaOH}$ 243, 302 and 335 (infl.) nm (ε 16,900, 8600, 6800), was unaltered in acid. Its bathochromic shift in alkali shows the alkaloid to be phenolic. The NMR-spectrum (100 MHz in pyridine-D_5)[4] showed that the molecule was a true dimer having 2 CH_2CH_3 (triplet, 0.67 δ, J = 7 HZ) and 2 N–$COCH_3$ (singlet, 2.33 δ) groups and 2 protons in position 2 of an N-acylaspidospermidine skeleton (broad quartet, 4.20 δ). Only 2 aromatic protons were present and these appeared as a singlet at 7.11 δ. Acetylation with acetic anhydride-pyridine (24 h at 25 °C, 15 min at 90 °C) gave a tetra-*O*-acetate, m.p. 135–140°, whose mass spectrum showed M^+, 878 with successive losses of ketene to give peaks at 836, 794, 752 and 710. The lower region of the mass spectrum showed doubly charged ions of the same masses, but was otherwise similar to that of the original alkaloid. Taking these facts into account, the mass spectral fragmentation is in entire accord with structure III. M-28, M-42, M-43, m/e 124 (base peak), 138 and 152 indicated an N-acetyl aspidospermidine structure, but the indolic peak found at m/e 162 in the spectrum of I is shifted to m/e 516 in both III and its tetraacetate. The difference in mass corresponds to substitution by a unit of structure I (with loss of 2 hydrogen atoms) and this peak is assigned structure (a). 2 minor peaks at m/e 160 and 190 are interpreted as due to the aliphatic portion (b)[5].

The alkaloid (I) has aromatic proton absorptions at 6.76 and 7.17 δ (J = 8 Hz). In the dimer, the absorption at higher field is absent. This can be attributed to the C-15 proton and the link between the 2 halves is thus placed at C-15, 15′ in III. Comparison of the UV-spectrum of the dimeric alkaloid with that of I shows a substantial increase in intensity as well as a bathochromic shift, indicating considerable conjugation of the aromatic rings. Models show that the degree of coplanarity necessary for such conjugation could only be achieved if the linkage were 15-15′[6].

Zusammenfassung. In *Aspidosperma melanocalyx* wurde N-Acetyl-16, 17-dihydroxy-aspidospermin (Desmethyl-aspidocarpin) gefunden sowie dessen durch oxydative Dimerisierung entstandenes Produkt. Die Strukturaufklärung basiert auf physikalisch-chemischen Methoden.

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Direct Synthesis of *p*-Methoxybenzyl Carbazate and *p*-Methoxybenzyloxycarbonylamino Acids Using *p*-Methoxybenzyl Chloroformate as Reagent[1]

Use of *p*-methoxybenzyloxycarbonyl [Z(OMe)] as a protective group in peptide synthesis was revived by the finding of Weygand and Hunger[2] in 1962 that the group can be removed easily with trifluoroacetic acid in the presence of anisole. The protective group can be introduced into amino acids with *p*-methoxybenzyl azidoformate[2], *p*-methoxybenzyl *p*-nitrophenyl carbonate[2], and *p*-methoxybenzyl 1-piperidyl carbonate[3]. However, a more convenient method should be developed for extensive use of this protective group.

This paper shows that *p*-methoxybenzyl chloroformate, which is easily prepared from anisyl alcohol and phosgene, can be used as a reagent to synthetize *p*-methoxybenzyl carbazate and Z(OMe)-amino acids directly by the Schotten-Baumann reaction. Practical conditions for synthesis of the chloroformate are also reported.

Materials and methods. A solution of anisyl alcohol (138 g, 1 mol) in dry ether (700 ml) was added dropwise with stirring to a solution of phosgene (200 g, 2 mol) in dry ether (1 l) over a period of 30 min at 0 °C. Then, the solution was concentrated to about 400 ml under reduced pressure in an ice-salt bath, and the resulting ether solution was used in following reactions as a solution of *p*-methoxybenzyl chloroformate without further purification.

This *p*-methoxybenzyl chloroformate solution (1 mol) was added drop-wise to a suspension of 80% hydrazine hydrate (625 g, 10 mol) in chloroform (1 l) at 0 °C over a period of 90 min with vigorous stirring. Stirring was continued for an additional 30 min at room temperature. The product was extracted with chloroform after addition of 2*N* NaOH (500 ml). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated to a residue, which was recrystallized from chloroform and *n*-hexane; the yield of *p*-methoxybenzyl carbazate was 167 g (85.2% from anisyl alcohol), m.p. 76 to 77 °C; reported m.p. 71–74 °C[2]. Anal. Found: C, 54.96; H, 6.19; N, 14.26%. Calcd. for $C_9H_{12}O_3N_2$: C, 55.09; H, 6.17; N, 14.28%.

[1] Presented at the 6th Symposium on Peptide Chemistry at Kyushu University, Fukuoka (Japan), 23 November 1968.

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Yields and physical constants of Z(OMe)-amino acids

Z(OMe)-amino acid	Organic solvent added	Medium for recrystallization	Yield %	Melting point °C	Optical rotation $[\alpha]_D$	Temperature °C
L-Phenylalanine DCHA	THF	EtOH-*n*-hexane	85.8	165–167.5	+ 24.5 (*c* 2.0, MeOH)	28
	none		76.0	165–167	+ 24.5 (*c* 2.04, MeOH)	28
				[157–159	+ 22.6 (*c* 1.82, MeOH)	24][a]
L-Phenylalanine[b]	THF	AcOEt-*n*-hexane		91–92.5	+ 5.2 (*c* 2.09, AcOH)	22
				[83–85	+ 5.7 (*c* 2.37, AcOH)	24][a]
Glycine	*i*-PrOH	AcOEt-*n*-hexane	84.7	94.5–95.5 [94–96][a]		
L-Alanine[b]	THF	AcOEt-*n*-hexane	96.3	80–82	− 12.4 (*c* 3.08, AcOH)	26
				[74–75	− 11.9 (*c* 3.11, AcOH)	23][a]
L-Valine[b,c]	THF	benzene-*n*-hexane	78.8	59–62 [oil][d]	− 1.3 (*c* 3.03, MeOH)	25
L-Leucine DCHA	*i*-PrOH	EtOH-*n*-hexane	80.0	164.5–165.5	− 6.2 (*c* 2.09, MeOH)	25
				[162	− 6.67 (*c* 2.1, MeOH)	24][a]
L-Isoleucine DCHA[e]	THF	EtOH-*n*-hexane	81.1	145–146	+ 3.5 (*c* 1.97, MeOH)	28
L-Threonine DCHA[f]	*i*-PrOH	EtOH-*n*-hexane	57.9	186–188(d)	+ 7.1 (*c* 2.0, MeOH)	28
L-Proline DCHA	*i*-PrOH	EtOH-*n*-hexane	94.3	150–151	− 25.1 (*c* 1.7, MeOH)	25
				[147.5–149	− 25.1 (*c* 1.68, MeOH)	28][a]
L-Methione DCHA	*i*-PrOH	EtOH-*n*-hexane	84.4	152.5–154.5	+ 3.4 (*c* 2.13, MeOH)	24
				[152–153	+ 3.35 (*c* 2.69, MeOH)	21][a]
S-*p*-Methoxybenzyl-L-cysteine[b,g]	*i*-PrOH	AcOEt-*n*-hexane	78.2	84–86	− 47.6 (*c* 2.24, MeOH)	25
O-Benzyl-L-tyrosine[h]	THF	AcOEt	82.9	97.5–98.5	+ 3.1 (*c* 0.88, AcOH)	25
L-Glutamine	*i*-PrOH	EtOH-*n*-hexane	70.0	144.5–145.5(d)	− 11.2 (*c* 1.0, MeOH)	27
				[143–144	− 10.8 (*c* 1, MeOH)	25][i]
L-Asparagine	THF	EtOH-*n*-hexane	41.3	159.5–160.5(d)	− 5.8 (*c* 1.15, MeOH)	25
				[158–159	− 5.3 (*c* 1, MeOH)	25][j]
L-Glutamic acid	THF	AcOEt-*n*-hexane	84.0	112.0–113.0(d)	− 5.7 (*c* 1.98, AcOH)	24
				[110–111(d)	− 5.55 (*c* 1.81, AcOH)	25][a]
ε-Carbobenzoxy-L-lysine DCHA	*i*-PrOH	EtOH-*n*-hexane	71.4	133–135	+ 4.4 (*c* 1.95, MeOH)	25
				[133–135	+ 4.4 (*c* 1.59, MeOH)	26][a]
γ-Benzyl-L-glutamate[b,k,l]	*i*-PrOH	AcOEt-*n*-hexane	71.4	87–88.5	− 10.1 (*c* 2.06, EtOH)	18
N^G-nitro-L-arginine[m]	none	EtOH-*n*-hexane	69.3	148–149.5(d)	− 9.3 (*c* 2.1, pyridine)	18

DCHA = dicyclohexylamine; MeOH = methanol; EtOH = ethanol; *i*-PrOH = iso-propanol; THF = tetrahydrofuran; AcOEt = ethyl acetate; AcOH = acetic acid; (d) = melts and decomposes. [a] The same literature referred as [2]. [b] Crystallized after extraction from the DCHA salt. [c] Anal. Found: C, 59.80; H, 6.73; N, 4.89%. Calcd. for $C_{14}H_{19}NO_5$: C, 59.77; H, 6.81; N, 4.98%. [d] T. KATO, M. KONDO, M. OHNO and N. IZUMIYA, Bull. chem. Soc. Japan *38*, 1202 (1965). [e] Anal. Found: C, 67.82; H, 9.62; N, 5.84%. Calcd. for $C_{27}H_{44}N_2O_5$: C, 68.03; H, 9.31; N, 5.88%. [f] Anal. Found: C, 64.40; H, 8.41; N, 5.96%. Calcd. for $C_{25}H_{40}N_2O_6$: C, 64.63; H, 8.68; N, 6.03%. [g] Anal. Found: C, 59.38; H, 5.77; N, 3.46; S, 7.71%. Calcd. for $C_{20}H_{23}NO_6S$: C, 59.24; H, 5.72; N, 3.45; S, 7.91%. [h] Anal. Found: C, 69.47; H, 5.76; N, 3.14%. Calcd. for $C_{25}H_{25}NO_6$: C, 68.95; H, 5.79; N, 3.22%. [i] E. SCHRÖDER and E. KLIEGER, Justus Liebigs Annln Chem. *673*, 196 (1964). [j] E. SCHRÖDER and E. KLIEGER, Justus Liebigs Annln Chem. *673*, 208 (1964). [k] 2*N* Na_2CO_3 was used as base. [l] Anal. Found: C, 62.81; H, 5.95; N, 3.41%. Calcd. for $C_{21}H_{23}NO_7$: C, 62.83; H, 5.78; N, 3.49%. [m] Anal. Found: C, 46.84; H, 5.59; N, 18.22%. Calcd. for $C_{15}H_{21}N_5O_7$: C, 46.99; H, 5.52; N, 18.27%.

The general procedure for the synthesis of Z(OMe)-amino acids is as follows: A solution of *p*-methoxybenzyl chloroformate (containing 0.125 mol, calculated from the anisyl alcohol content), prepared as described above, and 2*N* aqueous NaOH (about 50 ml) were added alternately to a mixture of amino acid (0.10 mol), 2*N* NaOH (50 ml), and tetrahydrofuran (50 ml) at 0 °C over a period of 30 min. The reaction mixture was agitated vigorously and was kept at pH 9–10 during this period. After addition of the reagents, stirring was continued for a further 1 h at room temperature. Then, tetrahydrofuran was removed by distillation under reduced pressure at pH 7. The residual solution was washed with ether and acidified to pH 2 with 2*N* HCl[4]. The oil which separated was extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4[5], concentrated to a residue, and recrystallized from a suitable solvent system.

[4] Z(OMe)-L-glutamine and L-asparagine, which crystallized at this stage, were collected by filtration and purified.

[5] Z(OMe)-N^G-nitro-L-arginine was soluble in ethyl acetate in a presence of water, and it began to crystallize out over the desicant as soon as the water was absorbed. Therefore, the desicant should be removed promptly as soon as excess water disappeared.

Results and discussion. Preparation of *p*-methoxybenzyl chloroformate was first reported by JONES and YOUNG[3]. We reinvestigated their reaction conditions using Silica gel G thin-layer chromatography with benzene as the developing solvent. Reaction between phosgene and anisyl alcohol in ether was found to be very fast even at below 0 °C. The stability of the chloroformate formed was as follows: (1) It was stable at below 0 °C when diluted with ether to about 1 mol/400 ml. (2) In more concentrated solution, the chloroformate was less stable and evolution of CO_2 was observed even at below 0 °C. (3) It decomposed gradually in dilute etherial solution, even at −10 °C, on addition of chloroform.

Finally, practical conditions for the preparation of the chloroformate were established as described above. The chloroformate solution should be stored in a deep freezer and used within a few days.

In the Schotten-Baumann reactions with amino acids, addition of water-soluble solvents, such as tetrahydrofuran or a lower-alkyl alcohol, increased the reaction rate, as in the same reactions with *t*-alkyloxycarbonyl chloride[6], but addition of these materials was not essential. The products which did not crystallize at this stage were obtained as dicyclohexylamine salts. Then, after reextraction with ethyl acetate, some of them were recovered as crystals of free Z(OMe)-amino acids by shaking their salts with NH_2SO_4. The Z(OMe)-amino acids synthesized in this way are shown in Table 1.

This procedure seems especially suitable for large-scale preparation, although good results have not yet been obtained with serine.

Zusammenfassung. *p*-Methoxybenzyloxycarbonylhydrazid und *p*-Methoxybenzyloxycarbonylaminosäuren konnten durch die Schotten-Baumannsche Reaktion mit *p*-Methoxybenzyloxycarbonylchlorid als Reagens direkt synthetisiert werden. Die Reaktionsbedingungen für die Darstellung des Reagens wurden verbessert und seine Stabilität wurde untersucht.

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Beeinflussung der ATPase aminspeichernder Granula von Nebennierenmark und Milznerven durch Thallium

Bei der Thalliumintoxikation des Menschen kommt es in einem Teil der Fälle zu Blutdrucksteigerung und Tachykardie[1,2], die durch α- und β-Rezeptorenblocker antagonistisch beeinflussbar sind[3]. Dabei wurde eine erhöhte Ausscheidung von Noradrenalin, in geringerem Masse auch von Adrenalin und 3-Methoxy-4-hydroxymandelsäure, im Harn beobachtet[3,4]. Die Symptome lassen sich als ein «erhöhter Erregungszustand des sympathischen Nervensystems»[3] deuten. Eine tierexperimentelle Nachahmung dieser Veränderungen gelang uns in Versuchen an Ratten bisher nicht.

Verschiedene ATPasen werden durch Tl^+ (Rattenerythrozyten[5], Kaninchennieren[6]) und durch Tl^{+++} (Rattenleber-Mitochondrien[7]) stimuliert. Der Effekt des einwertigen Thalliums ist durch seine Ähnlichkeit mit dem gleichsinnig wirkenden K^+-Ion erklärbar[5,6]. Auch für die intrazelluläre Anreicherung von Thallium[8,9] wird seine Beförderung durch den ATP-abhängigen K^+-Transportmechanismus diskutiert[5].

Da Thallium ins Zellinnere aufgenommen wird, liegt ein möglicher Angriffspunkt in den aminspeichernden Organellen chromaffiner Zellen oder peripherer Nervenendigungen. Sie enthalten eine Mg^{++}-abhängige, durch Na^+ und K^+ nicht aktivierbare ATPase, der eine entscheidende Rolle beim Transport der Amine zugeschrieben wird[10,11]. Wir untersuchten deshalb die Wirkung ein- und dreiwertiger Thalliumionen auf die Granula-ATPasen des Nebennierenmarks (NNM) und der Milznerven von Rindern.

Die Brenzcatechinamin-Granula des NNM wurden nach TAUGNER und HASSELBACH[12], die der Milznerven nach BURGER et al.[11] (60 000-g-Sediment) durch Ultrazentrifugation präpariert. Die Partikeln wurden in 0,3 *M* Saccharose resuspendiert, so dass 10 ml 1 g NNM (175–390 μg Protein/ml Suspension) bzw. 3 g Milznerven (250–400 μg Protein/ml Suspension) entsprachen.

Die Inkubationsansätze zur Bestimmung der ATPase-Aktivität enthielten:

0,1 ml 0,001 *N*HCl (Kontrollen) bzw. $TlCl_3$ oder Tl_2SO_4 in 0,001 *N*HCl

0,3 ml $8{,}3 \times 10^{-3}$ *M* $MgSO_4$ in 0,4 *M* *Tris*-Acetat-Puffer pH 7,4

0,4 ml Granulasuspension

0,2 ml *Tris*-ATP (0,0125 *M* Na-freies ATP in 0,3 *M* *Tris*-Acetat-Puffer pH 7,4).

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