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Note

Esterification of amino acids with thionyl chloride acidified butanols for their gas chromatographic analysis

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The advantages in the preparation of amino acid methyl esters of using thionyl chloride–methanol, instead of HCl–methanol (methanol saturated by different amounts of hydrogen chloride gas), have been detailed[1–9]. Recently the efficiency of *n*-propyl esterification of amino acids was reported[10]. To our knowledge, there are no literature data on the utilization of thionyl chloride–butanols for the esterification of amino acids.

Our work was intended (i) to provide a suitable derivatization procedure for the rapid butyl esterification of amino acids with a good reproducibility in order to clarify the optimum conditions using thionyl chloride–butanols as esterification mixtures and (ii) to compare the efficiency of the suggested method to the classical procedure[11].

MATERIALS AND METHODS

Reagents

All reagents and standard amino acids were of analytical purity, obtained from Reanal (Budapest, Hungary).

Apparatus

A Chromatron G.C.H.F. 18.3 gas chromatograph (VEB Chromatron, Berlin, G.D.R.) equipped with a flame ionization detector and a 3 m × 3 mm I.D. stainless-steel column was used. Nitrogen was the carrier gas at a flow-rate of 60 cm^3/min. The column packing consisted of 3% SP-2250 on Supelcoport (80–100 mesh) (Supelco, Bellefonte, PA, U.S.A.). The column temperature was increased from 100 to 310°C at 12 °C/min. The temperatures of the injector and detector were 260 and 350°C, respectively.

Esterification

A 1–2 cm^3 volume of the 1 *M* hydrochloric acid stock solution of the amino acids (each 1.5–5 mg/cm^3) was pipetted into a 25-cm^3 vessel (the ground joint of which can be fitted to a vacuum distillation device and to a reflux condenser, respectively) and evaporated to dryness under vacuum. To the residue, 1 cm^3 of *n*- or

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isobutanol was added containing 1 *M* of thionyl chloride. The reflux condenser was then fitted and the apparatus placed in a water-bath. Esterification took place at 100°C for 60 min. After cooling to room temperature, the solution was evaporated under vacuum, in a water-bath at 60°C, to a syrupy substance. The residue was quantitatively transferred with 5 × 0.1 cm^3 dichloromethane into a 2-cm^3 Pierce Reacti-Vial. A 0.5-cm^3 volume of trifluoroacetic anhydride was added and the acylation was carried out for 10 min at 150°C. A stock solution of 1 cm^3 was prepared, 5–10 μl aliquots of which were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Our model esterifications performed with the thionyl chloride–butanol mixtures and characteristic representatives of amino acids provided the following results.

(1) As to the optimum esterification time, with 1 mol thionyl chloride containing *n*- or isobutanol, respectively, to be on the safe side, we used 60 min (Tables I and II). *n*-Butyl esterifications were complete within 10 min, isobutyl esterification within 30 min, with the following exceptions: *n*-butyl esterification of valine, leucine and tryptophan and isobutyl esterification of histidine became quantitative after 40 min. Note: the prolonged reaction time necessary for the quantitative derivatization of valine, leucine, tryptophan and histidine is independent of the quality of the esterification agent applied.

(2) In order to select the most advantageous derivatization conditions, *i.e.*, the optimum molar ratios of thionyl chloride to butanols (Table I), the concentration of thionyl chloride was varied between 0.5 and 4.0 *M* (using *n*-butanol) and 0.5 and 1.5 *M* (in the case of isobutanol), respectively.

The *n*-butyl esterifications are quantitative in the concentration range of 0.75–4.0 *M*. Nevertheless, the concentration of thionyl chloride recommended is in the range of 0.75–1.5 *M*, since (i) when using a thionyl chloride concentration higher than 1.5 *M* the methionine was eluted in two peaks, and (ii) the stock solution of the derivatized amino acids became yellowish, probably due to side reactions (Table I, A). In the thionyl chloride–isobutanol system, thionyl chloride concentrations of 0.75 and 1.0 *M* can be utilized for analyses, since (i) in the case of 0.5 *M* the esterification of valine was not quantitative (Table I, B) and (ii) interfering peaks were detected at >2 *M*.

(3) The most advantageous volumes of the 1 *M* thionyl chloride containing butanols were also investigated. In both cases, using the thionyl chloride–*n*-butanol or –isobutanol mixtures, 0.5–2.0 cm^3 can be applied. We propose the use of 1 cm^3.

(4) The reproducibility of the determination of different amounts of amino acids esterified by the suggested method is summarized in Table II.

(5) Comparing the peak area responses and the analytical reproducibilities of the N-trifluoroacetyl, *n*- or isobutyl esters prepared by the suggested method with those obtained by the classical method[11] (Table I), the following conclusions could be drawn. (i) The peak area responses and the analytical reproducibilities, independently of the esterification agent used, are the same. (ii) The advantages of our esterification method are realized in the direct preparation of the reactant and in the fact that 1–2 cm^3 of the thionyl chloride–butanol mixture result in quantitative esterification of 10–15 mg of amino acids. In contrast, the classical prescription[12] requires 1.5 cm^3 hydrochloric acid–*n*-butanol for 1 mg amino acid.

TABLE I

ESTERIFICATION EFFICIENCY OF AMINO ACIDS USING *n*-BUTANOL (A) OR ISOBUTANOL (B) AS A FUNCTION OF THE CONCENTRATION OF THIONYL CHLORIDE APPLIED (a) AND APPLYING 6% (w/w) HYDROCHLORIC ACID-CONTAINING BUTANOLS (b), ACCORDING TO THE CLASSICAL METHODS[11,12]

Esterification time: 60 min. Injected amounts: 20–30 μg of each amino acid. All peak areas listed represent the mean of at least three (a) or four (b) measurements. Data in parentheses have been omitted from the means.

	Thionyl chloride (M)		*Peak area obtained, equivalent to 1 μg of substance*														
			Alanine	*Glycine*	*Valine*	*Leucine*	*Cysteine*	*Hydroxy-proline*	*Proline*	*Methionine*	*Aspartic acid*	*Phenyl-alanine*	*Glutamic acid*	*Lysine*	*Arginine*	*Tryptophan*	*Histidine*
(a)	0.5	A	(181)	(124)	(168)	(130)		(296)	(193)	(105)	(183)	(197)	(186)	(137)	(78)	(192)	(132)
	0.75		331	205	362	306		496	359	251	436	304	305	246	124	237	212
	1.0		333	218	366	281		508	360	259	426	304	355	256	115	206	248
	1.5		364	216	375	312		514	377	255	439	336	325	256	118	220	212
	2.0		331	197	404	297		475	340	231*	415	304	323	246	118	216	210
	4.0		326	214	376	312		501	369	251*	454	312	333	265	119	219	247
	Mean		337	210	377	302		499	361	249	434	312	337	254	119	220	226
	S.D.		15.3	8.8	16.4	13.1		15.0	13.8	10.9	14.6	13.9	14.9	10.2	3.3	11.2	19.8
	S.D. (%)		4.5	4.2	4.4	4.3		3.0	3.8	4.4	3.4	4.4	4.3	4.0	2.8	5.1	8.8
(b)	Mean		338	200	383	304	304	301	483	384	235	453	352	361	277	114	240
	S.D.		8.5	13.2	22.8	11.2	26.7	16.1	14.9	10.5	20.6	5.3	14.7	14.2	3.2	8.3	
	S.D. (%)		2.5	6.6	5.9	3.7	8.9	3.3	3.9	4.5	4.5	1.5	4.0	5.1	2.8	3.4	
(a)	0.5	B	377	316	(363)	417	203	390	609	214	604	646	605	265	130		191
	0.75		382	308	398	442	203	392	619	205	623	664	597	270	135		199
	1.0		379	315	396	418	195	388	636	226	617	643	607	274	131		191
	1.5		386	(284)	421	419	(125)	382	620	224	600	633	(531)	268	125		192
	Mean		381	313	405	424	200	388	621	217	611	647	603	269	130		193
	S.D.		3.9	4.3	13.8	12.0	4.6	4.3	11.2	9.7	10.8	12.9	5.3	3.8	4.1		3.4
	S.D. (%)		1.0	1.3	3.4	2.8	2.3	1.1	1.8	4.4	1.8	2.0	0.9	1.4	3.2		1.8
(b)	Mean		378	301	421	409	200	399	640		610	640	589	250	130	169	
	S.D.		11.5	14.4	8.2	8.3	3.2	7.8	16.8		5.5	16.4	18.9	5.7	2.0	8.3	
	S.D. (%)		3.0	4.8	1.9	2.0	1.6	1.9	2.6		0.9	2.6	3.2	2.2	1.5	4.9	

* Realized in two peaks.

TABLE II

REPRODUCIBILITY OF THE DETERMINATION OF DIFFERENT AMOUNTS OF AMINO ACIDS AS THEIR N-TRIFLUOROACETYL *n*-BUTYL (A) AND ISOBUTYL (B) ESTERS

Esters were prepared with 1 cm³ of butanol containing 1 *M* thionyl chloride; esterification time 60 min. All peak areas listed represent the mean of at least three measurements.

Amino acid	*A*					*B*			
	Injected amount (µg)	*Peak area per 1 µg*	*Mean*	*R.S.D.*	*R.S.D. (%)*	*Peak area per 1 µg*	*Mean*	*R.S.D.*	*R.S.D. (%)*
Alanine	44.52	361	359	4.1	1.1	393	389	11.9	3.0
	22.26	361				399			
	11.13	354				376			
Glycine	44.48	184	194	8.1	4.7	301	299	2.1	0.7
	22.24	198				300			
	11.12	201				297			
Valine	45.12	400	399	9.5	2.4	422	419	3.6	0.9
	22.56	408				420			
	11.28	389				415			
Leucine	41.60	304	310	5.1	1.7	419	420	3.1	0.7
	20.80	311				417			
	10.40	314				423			
Cysteine	40.28	313	309	4.6	1.5	180	190	8.4	4.4
	20.14	304				194			
	10.07	310				195			
Hydroxy-proline	40.00	458	472	14.0	3.0	388	391	4.4	1.1
	20.00	486				389			
	10.00	471				396			
Proline	67.24	379	379	9.5	2.5	647	639	7.5	1.2
	33.62	388				639			
	16.81	369				632			
Methionine	29.56	229	240	14.0	5.8	253	250	4.2	1.7
	14.78	236				245			
	7.39	256				251			
Aspartic acid	74.64	386	386	1.6	0.4	600	610	9.0	1.5
	37.32	385				615			
	18.66	388				616			
Phenyl-alaine	74.44	356	352	15.8	4.5	616	630	13.7	2.2
	37.22	335				645			
	18.61	366				628			
Tyrosine	36.20	376	376	3.5	0.9				
	18.10	373							
	9.05	380							
Glutamic acid	110.32	375	364	10.5	2.9	583	591	6.9	1.2
	55.16	354				595			
	27.58	364				595			

(*Continued on p. 438*)

TABLE II (*continued*)

Amino acid	*A*					*B*			
	Injected amount (µg)	*Peak area per 1 µg*	*Mean*	*R.S.D.*	*R.S.D. (%)*	*Peak area per 1 µg*	*Mean*	*R.S.D.*	*R.S.D. (%)*
Lysine	59.80	241	232	9.6	4.1	236	240	4.0	1.7
	29.90	234				244			
	14.95	222				240			
Arginine	51.64	118	116	4.7	4.0	118	116	5.3	4.5
	25.82	111				110			
	12.91	120				120			
Tryptophan	44.04	221	225	7.8	3.5	201	207	5.5	2.6
	22.02	234				210			
	11.01	220				211			
Histidine	36.20	200	207	11.5	5.5	177	171	13.6	7.9
	30.72	200				180			
	15.36	220				155			

This work in addition to our earlier studies forms part of our comprehensive research concerning the development of new and fast analytical methods[13–18] to determine the main constituents of natural matrices by gas chromatography.

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