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Chromatographic and Chromato–Mass Spectral Characterization of Amino Acids Derivatives Formed via the Interaction with Dimethyl Acetal of Dimethylformamide

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Abstract—A series of amino acids have been converted into the derivatives via interaction with dimethylformamide dimethyl acetal for gas chromatography identification. The derivatives have been for the first time characterized with mass spectra and retention indices corresponding to the standard nonpolar polydimethylsiloxane stationary phases. Basic features of mass spectroscopic fragmentation of the derivatives have been stated; the rules for interpretation of their gas chromatography retention indices have been figured out, including the additive scheme elements.

Keywords: amino acid, dimethylformamide, dimethyl acetal, mass spectrum, gas chromatography retention index, identification

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Organic compounds containing several functional groups with active hydrogen atoms are often insufficiently volatile and thermally stable to be directly analyzed by means of gas chromatography. Analysis of such compounds is possible after conversion in the more volatile derivatives via substitution of the active hydrogen atoms with less polar covalently bound fragments [1–3].

Amino acids present a typical example of compounds requiring the modification for performing their gas chromatography analysis. The direct gas chromatography analysis of free amino acids has been reported in the literature (for example, [4]); however, such studies seem to be faulty [5]. A few methods for amino acids modification are known [3, 6], the preferred one being the single-stage substitution of hydrogen atoms of the COOH and NH_2 groups under the action of dimethylformamide dialkyl acetals $(CH_3)_2N–CH(OR)_2$ (R = CH_3, C_2H_5, C_3H_7, *tert*-C_4H_9, etc.) [7]. Using the simplest representative of this class, dimethylformamide dimethyl acetal, is the most convenient in view of further gas chromatography analysis. The pioneering examples of the described approach have been given in [8–10].

Dimethylformamide dialkyl acetals act as alkylating agents with respect to many compounds containing active hydrogen atoms (carboxylic acids, phenols, thiols, etc.) [7]. The corresponding reactions have been considered to yield the alkyl ester and dimethylformamide [11].

$$RCO_2H + (CH_3)_2N–CH(OCH_3)_2$$
$$\rightarrow RCO_2CH_3 + CH_3OH + (CH_3)_2N–CHO.$$

Our studies, however, revealed that dimethylformamide (the retention index for standard nonpolar polydimethylsiloxane stationary phases being of 749±16) was not detected in noticeable amount in the starting reagent (the major component content being of ≈93 %) and in the reaction mixtures. Hence, we suggested that the intermediate monoalkyl acetals can be decomposed into dimethylamine and (in the case of DMF dimethyl acetal) methyl formate.

$$RCO_2H + (CH_3)_2N–CH(OCH_3)_2$$
$$\rightarrow RCO_2CH_3 + [(CH_3)_2N–CH(OH)OCH_3]$$
$$\rightarrow (CH_3)_2NH + HCO_2CH_3.$$

Retention indices of the both products for the standard nonpolar phases are low (dimethylamine 383±15, methyl

formate 386±10); therefore, they did not interfere with detection of the target reaction products and were not separated from the solvent signal (CH_2Cl_2, 515±7).

DMF dialkyl acetals act as synthetic equivalents of carbonyl compounds in the reactions with primary amines, forming the Schiff's bases (1,1-dialkyl formamidines or dimethylaminomethylene N-DMAM derivatives of primary amines) [12].

$$RNH_2 + (CH_3)_2N–CH(OCH_3)_2 \rightarrow RN=CH–N(CH_3)_2 + 2CH_3OH.$$

Secondary amines gave the *N*-formyl derivatives under the similar conditions [10]. Such reactions are believed to involve formation of the corresponding dialkyl acetals via transamination, followed by hydrolysis.

$$R_2NH + (CH_3)_2N–CH(OCH_3)_2 \rightarrow (CH_3)_2NH + [R_2N–CH(OCH_3)_2] \rightarrow R_2N–CHO.$$

Majority of amino acids contain primary amino groups and therefore form the derivatives of their methyl esters (N-DMAM-Me) in the reaction with DMF dimethyl acetal [2, 7]. Such derivatives have been used in gas chromatography analysis for about 30 years but have not been systematically characterized with the mass spectral and chromatographic (retention indices on the standard stationary phases) parameters so far (in particular, the relevant data have been absent in the latest version of NIST mass spectra database [13]), meaning that each analysis of amino acids in the form of the N-DMAM-Me derivatives should include modification of the reference amino acids samples, whereas the analytical devices are used as simple comparators. Such state of the art does not comply with the potential of contemporary chromato–mass spectrometry; therefore, in this work we obtained the reference mass spectra of the products of amino acids interaction with DMF dimethyl acetals and determined the gas chromatography retention indices of the products on the standard nonpolar stationary phases. The obtained data enable analysis of other amino acids as well.

The possibility to prepare the derivatives under the action of DMF dimethyl acetal was verified using sets of acids (decanoic, butanedioic, and citric ones) and amines (aniline, benzylamine, and monoethanolamine) as examples. Direct gas chromatography analysis was possible in the cases of monocarboxylic acids and primary amines, allowing for determination of the conversion degree from the areas of peak corresponding to the reaction substrates and products. Table 1 lists the gas chromatography retention indices (*RI*) of the substrates and the products, including the experimental values RI_{exp} determined in this work, the reference values RI_{ref}, and the reference mass spectra. In all the cases the substrates signals were not detected in the reaction mixture chromatogram, thus confirming the quantitative modification of the substrates in the corresponding derivatives.

It is generally believed [3] that the presence of the *p*–π conjugation (–N=CH–N<) in the N-DMAM derivatives explains the high intensity of the molecular ion signals in the mass spectra. However, this is only true in the cases of the presence of the only conjugation system in the molecule (i.e., in the cases of the aromatic amino acids derivatives Ar–N=CH–N<); for example, $I_{rel}(M)$ 80% for the aniline derivative. The presence of several alternative centers of the charge localization in the molecular ions regularly decreases the $I_{rel}(M)$ values [16] (11% for the monoethanolamine derivative and ~0.1% for certain amino acids). The strongest signals in the mass spectra were those at *m*/*z* 44 (NMe_2) and [*M* – 44] or (in certain cases) at *m*/*z* = 91 (C_7H_7) and *m*/*z* = 71 (Me_2NCHN) (the benzylamine derivative) and *m*/*z* 85 [*M* – CH_2OH] (the ethanolamine derivative) (Table 1).

Gas chromatography retention indices of all the prepared N-DMAM derivatives were determined using the standard nonpolar polydimethylsiloxane stationary phase BPX-1 and the more polar RTX-5 phase containing 5% of phenyl groups (the phases similar to the latter ones are referred to as "semi-standard" [13]) (Table 1).

The average difference between the *RI* values of the prepared N-DMAM derivatives determined for the two stationary phases was of 14±4 (Table 1), typical of the low-polar compounds. Another important gas chromatography parameter was the average difference between *RI* values of the substrate and its N-DMAM derivative. The latter parameter reflected the changes of the chromatography retention properties due to the substrate modification and gave a base for the simple additive scheme allowing for estimation of the retention index of the reaction product from the relevant substrate parameters [17]. The data in Table 1 show that for the scheme below the discussed value was of 396±16.

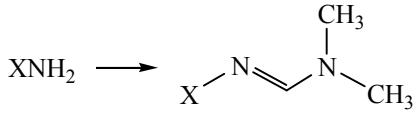


Table 1. Mass spectra and gas chromatography retention indices of products of the interaction of selected carboxylic acids and primary amines with dimethylformamide dimethyl acetal[a]

Substrate (*RI*)	Product		
	RI_{exp}	RI_{ref} [13–15]	$m/z \geq 40$ ($I_{rel} \geq 2\%$)
Decanoic acid (1378±10)	*1313±1*	1312±7	Not required. Retention index is sufficient for identification
Butanedioic acid (no data)	*1008±3*	1004±8	Not required. Retention index is sufficient for identification
Citric acid (no data)	*1424±2*	1424±3	Not required. Retention index is sufficient for identification
Aniline (958± 13)	*1366±2* 1384±3	1391±10	149 (8), 148 (80) [*M*], 147 (49), 134 (3), 133 (31), 132 (5), 131 (4), 120 (14), 119 (2), 118 (3), 107 (5), 106 (54), 105 (7), 104 (33) [$M - N(CH_3)_2$], 92 (2), 91 (6), 79 (10), 78 (11), 76 (5), 75 (2), 74 (8), 73 (10), 65 (3), 64 (2), 63 (2), 57 (7), 52 (4), 51 (34), 50 (8), 45 (22), 44 (100) [$N(CH_3)_2$], 43 (4), 42 (25), 41 (3), 39 (4)
Benzylamine (994±10)	*1396±2* 1407±2	1408	163 (5), 162 (46) (M), 161 (9), 147 (9), 134 (3), 132 (2), 120 (8), 119 (2), 118 (6), 117 (3), 106 (13), 104 (2), 103 (2), 92 (6), 91 (65) [$C_6H_5CH_2$], 90 (5), 89 (7), 85 (5), 84 (4), 83 (2), 81 (2), 79 (3), 77 (4), 71 (26) [$(CH_3)_2NCHN$], 65 (17), 63 (4), 58 (4), 57 (18), 51 (5), 50 (2), 46 (6), 45 (17), 44 (100) [$N(CH_3)_2$], 43 (3), 42 (34), 41 (4), 39 (6)
2-Aminoethanol (675±23)	*1052±2* 1064±2	No data	116 (11) (M), 98 (3), 86 (4), 85 (61) [$M - CH_2OH$], 72 (3), 71 (3), 58 (2), 57 (4), 46 (4), 45 (14), 44 (100) [$N(CH_3)_2$], 43 (6), 42 (34), 41 (7)

[a] Retention indices determined for the standard nonpolar phase BPX-1 are given in italics; other values correspond to the RTX-5 phase containing 5% of phenyl groups.

According to that estimation, the expected *RI* value of the N-DMAM derivative of 2-phenylethylamine (*RI* = 1079±11) was of 1475±19.

Table 2 gives the retention indexes and the reference mass spectra of the products of modification of 15 amino acids $RCH(NH_2)CO_2H$ via the reaction with DMF dimethyl acetal. The scheme of the conversion for the substrates containing primary amino groups is given below.

$$RCH(CO_2H)NH_2 + (CH_3)_2N{-}CH(OCH_3)_2 \rightarrow RCH(CO_2CH_3){-}N{=}CH{-}N(CH_3)_2.$$

In certain cases, the reaction mixtures contained the non-identified components or the methyl esters (for example, in the case of tryptophane).

Since the both functional groups of amino acids were involved in the reaction with DMF dimethyl acetal, the target products were designated as X-N-DMAM-Me (X being the code of the corresponding amino acid). In the cases of glycine and histidine, the reaction products were not detected owing to insolubility of the amino acids in the reaction mixture.

The only detected product of the reaction with threonine was assigned to the N-DMAM derivative of the primary amine rather than the starting amino acid derivative. Indeed, the mass spectrum contained the signals at m/z = 44, 70, and 71 corresponding to the $(CH_3)_2N{-}CH{=}N{-}$ fragment, but the *RI* value (1036±2) was lower than that for Ser-N-DMAM-Me (1233±2); therefore, the product could not be the threonine derivative. The component structure could be elucidated from the mass spectroscopy and chromatography data taking advantage of the algorithm discussed in [18]. To do so, the experimentally determined *RI* value should be reduced by the increment corresponding to the $-NH_2 \rightarrow -N{=}CH{-}N(CH_3)_2$ conversion, that gave the *RI* value of the starting primary amine, (1036±2) – (396±16) ≈ 640±16, the amine molecular mass being 55 Da lower than that of the N-DMAM derivative. The mass spectrum of the unknown component contained the strong signals at m/z 112 and (112 – 15) = 97; however, they could not be assigned to the $M^{+\cdot}$ and $[M - CH_3]^+$ ions, since the *RI* values of all the amines with M = 112 – 55 = 57 were much lower than 640±16. The only suitable solution was 1-aminopropanol-2 that could be formed via threonine decarboxylation. The refence *RI* value of the suggested solution (654±13) practically coincided with the estimation made (640±16, RTX-5); the agreement was

Table 2. Mass spectra and gas chromatography retention indices of products of the interaction of selected α-amino acids with dimethylformamide dimethyl acetal[a]

Amino acid (*M*)	*RI*(BPX-1)/*RI*(RTX-5)	Reaction product: $m/z \geq 40$ ($I_{rel} \geq 2\%$)
Glycine (75→144)	–	
Alanine (89→158)	1143±3/1176±2	Ala-N-DMAM-Me: 158 (6) [*M*], 100 (6), 99 (100) [$M - CO_2CH_3$], 98 (2), 83 (3), 72 (8), 71 (2), 59 (2), 58 (3), 57 (5), 56 (12), 55 (4), 54 (2), 46 (4), 45 (5), 44 (99) [NMe_2], 43 (4), 42 (22)
Serine (105→174)	–/1025±4	Not identified: 119 (0.3), 101 (11), 86 (4), 84 (3), 71 (12), 70 (100), 69 (16), 59 (7), 56 (5), 55 (2), 54 (2), 51 (2), 49 (2), 45 (3), 44 (10), 43 (15), 42 (50), 41 (9), 40 (9)
	–/1046±3	Not identified: 118 (3), 102 (14), 76 (3), 75 (100) [$CH(OMe)_2$], 74 (5), 59 (2), 58 (4), 47 (20), 44 (3), 43 (6), 42 (59), 41 (2)
	1233±2/1280±2	Ser-N-DMAM-Me: 173 (0.1) [*M* – H], 156 (18) [$M - H_2O$], 141 (2), 125 (2), 124 (13), 98 (3), 97 (29) [$M - H_2O - CO_2CH_3$], 96 (24), 95 (3), 84 (2), 81 (2), 72 (3), 71 (3), 68 (2), 57 (5), 56 (5), 55 (2), 54 (16), 53 (2), 52 (2), 45 (6), 44 (100) [NMe_2], 43 (9), 42 (34), 41 (4)
Proline (115→157)	1332±2/1389±2	Methyl ester of *N*-formylproline: 157 (5) [*M*], 129 (8), 106 (3), 99 (7), 98 (100) [$M - CO_2CH_3$], 86 (2), 77 (5), 73 (2), 71 (6), 70 (90) [$M - CO_2CH_3 - CO$], 69 (3), 68 (14), 55 (2), 51 (3), 50 (2), 45 (2), 44 (10), 43 (29), 42 (11), 41 (26), 39 (7), 38 (2)
Valine (117→186)	1264±2/1288±2	Val-N-DMAM-Me: 186 (4) [*M*], 144 (6), 143 (77) [$M - C_3H_7$], 142 (3), 128 (5), 127 (61) [$M - CO_2CH_3$], 115 (2), 100 (2), 86 (2), 84 (4), 83 (6), 82 (2), 73 (4), 72 (6), 71 (4), 69 (2), 68 (2), 59 (4), 58 (2), 57 (13), 56 (4), 55 (8), 46 (15), 45 (6), 44 (100) [NMe_2], 43 (4), 42 (21), 41 (8), 39 (3)
Threonine (119→188)	–/1036±2	N-DMAM derivative of 1-amino-2-propanol: 113 (5), 112 (66), 111 (7), 98 (3), 97 (40), 96 (2), 85 (6), 84 (8), 83 (7), 82 (4), 80 (3), 71 (10), 70 (24), 69 (6), 68 (29), 67 (6), 58 (5), 57 (8), 56 (25), 55 (2), 54 (4), 46 (5), 45 (14), 44 (100) [NMe_2], 43 (11), 42 (67), 41 (51), 40 (6), 39 (20), 38 (2)
Leucine (131→200)	1342±2/1380±2	Leu-N-DMAM-Me: 200 (0.1) [*M*], 157 (7), 156 (39) [$M - NMe_2$], 144 (9), 143 (18) [$M - C_4H_9$], 142 (7), 141 (73) [$M - CO_2CH_3$], 125 (2), 112 (15), 100 (2), 99 (20), 98 (3), 97 (6), 96 (2), 87 (2), 86 (2), 85 (41), 84 (9), 83 (5), 82 (2), 73 (5), 72 (11), 71 (5), 70 (5), 69 (3), 59 (2), 58 (2), 57 (9), 56 (6), 55 (5), 54 (5), 53 (2), 46 (13), 45 (6), 44 (100) [NMe_2], 43 (11), 42 (25), 41 (10), 39 (3)
Isoleucine (131→200)	1344±2/1380±2	Ile-N-DMAM-Me: 199 (0.1) [*M* – H], 157 (3), 156 (28) [$M - NMe_2$], 144 (7), 143 (74) [$M - C_4H_9$], 142 (4), 141 (39) [$M - CO_2CH_3$], 115 (2), 113 (3), 112 (7), 111 (2), 100 (2), 99 (2), 97 (2), 96 (2), 85 (5), 84 (7), 83 (5), 74 (2), 73 (28), 72 (6), 71 (5), 70 (3), 69 (7), 68 (3), 59 (2), 58 (2), 57 (8), 56 (3), 55 (3), 46 (14), 45 (6), 44 (100) [NMe_2], 43 (6), 42 (23), 41 (13), 39 (3)
Norleucine (131→200)	1390±2/1426±2	nor-Leu-N-DMAM-Me: 200 (0.2) [*M*], 157 (5), 156 (34) [$M - NMe_2$], 144 (2), 143 (14) [$M - C_4H_9$], 142 (6), 141 (73) [$M - CO_2CH_3$], 140 (3), 112 (5), 100 (2), 99 (8), 98 (3), 97 (4), 96 (2), 85 (15), 84 (5), 83 (4), 73 (7), 72 (6), 71 (7), 70 (2), 69 (4), 68 (2), 59 (2), 58 (2), 57 (7), 56 (4), 55 (4), 53 (3), 46 (10), 45 (6), 44 (100) [NMe_2], 43 (11), 42 (22), 41 (9), 29 (2)
Asparaginic acid (133→216)	1460±2/1516±2	Asp-N-DMAM-Me_2: 216 (10) [*M*], 185 (2), 184 (5), 158 (6), 157 (68) [$M - CO_2CH_3$], 156 (18), 155 (5), 143 (11), 125 (4), 116 (2), 115 (27), 100 (3), 99 (37), 98 (3), 97 (9), 87 (4), 84 (2), 83 (9), 74 (4), 73 (2), 72 (4), 71 (2), 70 (2), 69 (2), 59 (6), 57 (11), 56 (6), 55 (6), 54 (16), 46 (5), 45 (7), 44 (100) [NMe_2], 43 (5), 42 (25), 41 (3)

Table 2. (Contd.)

Amino acid (*M*)	*RI*(BPX-1)/*RI*(RTX-5)	Reaction product: $m/z \geq 40$ ($I_{rel} \geq 2\%$)
Lysine (146→270)	1961±2/1994±13	Lys-bis-N-DMAM-Me: 270 [*M*], 226 (3), 211 (3) [*M* – CO_2CH_3], 200 (3), 199 (8), 198 (4), 166 (5), 165 (4), 157 (6), 156 (2), 154 (2), 144 (3), 143 (6), 140 (4), 139 (19), 137 (2), 129 (2), 128 (31), 127 (17), 125 (4), 114 (4), 113 (4), 112 (18), 111 (9), 100 (3), 99 (15), 98 (4), 97 (6), 96 (3), 94 (3), 86 (13), 85 (29), 84 (11), 83 (5), 82 (3), 73 (15), 72 (10), 71 (11), 70 (100) [Me_2N–CN], 69 (7), 68 (4), 67 (3), 59 (2), 58 (7), 57 (14), 56 (6), 55 (3), 54 (2), 46 (34), 45 (8), 44 (82) [NMe_2], 43 (7), 42 (28), 41 (7)
Glutamic acid (147→230)	1578±2/1637±2	Glu-N-DMAM-Me_2: 230 (11) [*M*], 199 (10), 198 (4), 172 (4), 171 (48) [*M* – CO_2CH_3], 170 (20), 169 (4), 158 (2), 157 (29), 155 (11), 143 (12), 141 (2), 139 (8), 128 (2), 112 (9), 111 (100) [*M* – CO_2CH_3 – HCO_2CH_3], 100 (2), 99 (4), 98 (3), 97 (10), 96 (2), 95 (2), 85 (7), 84 (7), 83 (5), 82 (2), 74 (2), 73 (16), 72 (6), 71 (8), 70 (9), 69 (2), 68 (5), 59 (6), 58 (3), 57 (18), 56 (6), 55 (4), 54 (3), 46 (21), 45 (8), 44 (88) [NMe_2], 43 (7), 42 (38), 41 (12), 40 (2), 39 (3)
Methionine (149→218)	1581±2/1638±2	Met-N-DMAM-Me: 218 (5) [*M*], 203 (3), 186 (2), 174 (2), 171 (2), 160 (2), 159 (15) [*M* – CO_2CH_3], 158 (7), 157 (81) [*M* – CH_3SCH_2], 145 (3), 144 (40), 143 (21) [*M* – $(CH_2)_2SCH_3$], 128 (5), 115 (2), 113 (4), 112 (61), 111 (60) [*M* – CO_2CH_3 – CH_3SH], 104 (3), 100 (2), 99 (10), 98 (3), 97 (23), 87 (3), 85 (9), 84 (36), 83 (5), 75 (2), 74 (2), 73 (4), 72 (6), 71 (6), 70 (5), 69 (2), 68 (4), 63 (2), 62 (2), 61 (43) [CH_3SCH_2], 59 (3), 58 (4), 57 (18), 56 (5), 55 (3), 54 (4), 47 (3), 46 (9), 45 (11), 44 (100) [NMe_2], 43 (4), 42 (36), 41 (11), 40 (2), 39 (3)
Histidine (155→224)	–	
Phenylalanine (165→234)	1692±2/1732±2	Phe-N-DMAM-Me: 235 (4), 234 (27) [*M*], 176 (3), 175 (20) [*M* – CO_2CH_3], 162 (11), 161 (100) [*M* – $PhCH_2$], 134 (8), 132 (2), 131 (3), 121 (12), 120 (15), 119 (4), 118 (3), 117 (3), 105 (3), 104 (12), 103 (11), 102 (3), 99 (29), 92 (2), 91 (36), 90 (4), 89 (4), 80 (9), 79 (2), 78 (6), 77 (12), 76 (2), 73 (3), 72 (22), 51 (5), 46 (4), 45 (6), 44 (90) [NMe_2], 43 (4), 42 (27), 41 (2), 39 (2)
Arginine (174→298)	1842±2/1909±2	Arg-bis-N-DMAM-Me: 298 [*M*], 250 (1), 248 (1), 212 (5), 211 (2), 197 (2), 187 (2), 186 (13), 185 (4), 184 (32), 168 (2), 157 (6), 153 (2), 152 (5), 168 (2), 157 (6), 153 (2), 152 (5), 151 (4), 143 (6), 142 (7), 141 (8), 140 (4), 129 (3), 128 (28), 126 (6), 125 (28), 124 (6), 114 (3), 113 (3), 111 (5), 109 (3), 101 (3), 100 (36), 99 (46), 98 (63), 97 (17), 96 (6), 85 (22), 84 (6), 83 (11), 82 (7), 81 (2), 80 (6), 75 (2), 73 (23), 72 (41), 71 (27) [(Me_2NCHN], 70 (13), 69 (3), 68 (8), 67 (2), 59 (3), 58 (6), 57 (15), 56 (8), 55 (6), 54 (4), 53 (2), 46 (21), 45 (12), 44 (100) [NMe_2], 43 (22), 42 (37), 41 (9), 40 (8)
Tyrosine (181→250)	–/1987±4	Tyr-N-DMAM-Me: 250 [*M*], 191 (9) [*M* – CO_2CH_3], 144 (6), 143 (53) [$Me_2NCHNCHCO_2CH_3$], 107 (2), 96 (6), 91 (4), 84 (3), 83 (7), 79 (2), 72 (7), 71 (2), 68 (2), 58 (2), 57 (10), 56 (3), 53 (3), 46 (6), 45 (4), 44 (100) [NMe_2], 43 (3), 42 (22), 41 (3), 39 (3)
	1994±2/2038±5	Tyr-N-DMAM-Me_2: 264 [*M*], 205 (5) [*M* – CO_2CH_3], 144 (8), 143 (72) [$Me_2NCHNCHCO_2CH_3$], 133 (2), 122 (2), 121 (9), 103 (11), 100 (2), 91 (2), 84 (2), 79 (2), 78 (6), 77 (4), 76 (2), 74 (2), 73 (5), 72 (5), 71 (6), 64 (2), 57 (11), 56 (2), 53 (2), 51 (2), 46 (9), 45 (7), 44 (100) [NMe_2], 42 (21), 41 (3), 40 (2)

Table 2. (Contd.)

Amino acid (*M*)	*RI*(BPX-1)/*RI*(RTX-5)	Reaction product: $m/z \geq 40$ ($I_{rel} \geq 2\%$)
Tryptophane (204→273)	2161±9/–	Methyl ester of tryptophane: 218 [*M*], 202 (2), 201 (12), 181 (2), 170 (2), 169 (2), 155 (2), 131 (13), 130 (100) [(3-indolyl)CH_2], 128 (3), 115 (3), 103 (4), 102 (4), 78 (2), 77 (5), 75 (3), 44 (4), 43 (3)
	2327±12/2321±15	Trp-N-DMAM-Me: 273 [*M*], 214 (5) [*M* – CO_2CH_3], 169 (5), 145 (2), 144 (30), 143 (100) [$Me_2NCHNCHCO_2Me$], 142 (2), 131 (3), 130 (26), 129 (2), 128 (2), 117 (2), 116 (2), 115 (8), 112 (12), 107 (5), 103 (4), 102 (2), 100 (2), 89 (2), 84 (6), 83 (4), 77 (6), 73 (2), 72 (4), 58 (2), 57 (7), 45 (3), 44 (58) [NMe_2], 43 (2), 42 (20)
	–/2374±17	N-formyl-Trp-N-DMAM-Me: 301 [*M*], 242 (4) [*M* – CO_2CH_3], 169 (2), 144 (10), 143 (100) [$Me_2NCHNCHCO_2Me$], 142 (2), 130 (7), 115 (6), 107 (2), 103 (2), 102 (3), 83 (4), 77 (3), 72 (5), 57 (4), 46 (5), 45 (2), 44 (50) [NMe_2], 42 (12)

[a] ("–") Products not detected.

absolute after correcting for the *RI* difference for the BPX-1 and RTX-5 phases (14±4 index units). The signal at m/z = 112 could then be assigned to the $[M - H_2O]^+$ ions; such signals were found in the mass spectra of serine methyl ester and the Ser-N-DMAM-Me derivative (Table 2).

The average *RI*(RTX-5) – *RI*(BPX-1) difference for the N-DMAM derivatives of amino acids was of 46±14 index units (Table 2), meaning that those compounds were more polar than the N-DMAM derivatives of monofunctional primary amines. The molecular ion peaks were reliably detected (I_{rel} = 4–11%) only in the mass spectra of the alanine, valine, asparaginic acid, glutamic acid, methionine, and phenylalanine derivatives. In the cases of other amino acids, the I_{rel} of the signal of the derivative molecular ion was of 0.1–0.2%. Figure 1 gives a spectrum of nor-Leu-N-DMAM-Me as a representative example of the latter case.

One of the limitations of mass spectrometry analysis of amino acids and peptides is the complicated identification of the isomers, first of all, the leucine–isoleucine pair. Retention indexes of their N-DMAM derivatives were almost the same (1342±2 and 1344±2), but their mass spectra were significantly different: for leucine I_{rel} (m/z = 143) >> I_{rel} (m/z = 141), and for isoleucine the given relation was the opposite. On the contrary, the isoleucine–norleucine pair could not be distinguished using the given mass spectral feature, but their *RI* values were significantly

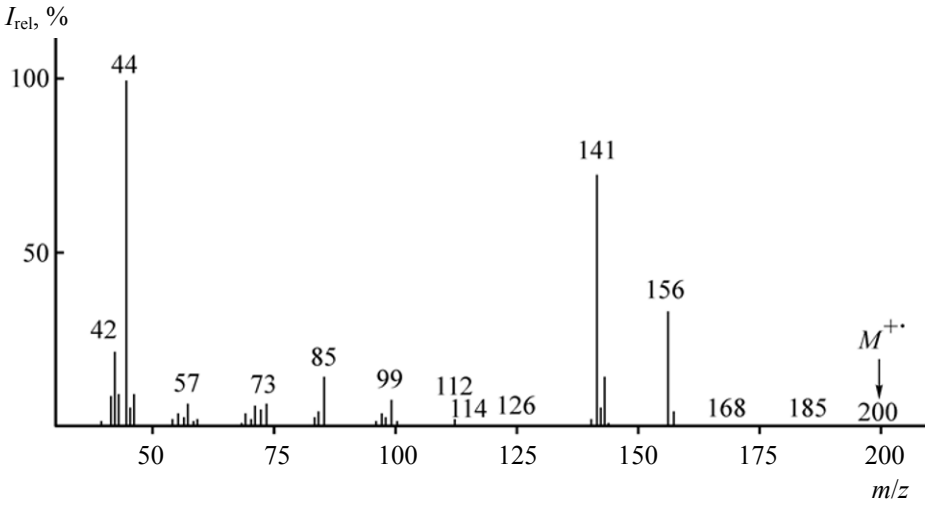


Fig. 1. Mass spectrum of N-DMAM derivative of norleucine methyl ester (*M* = 200).

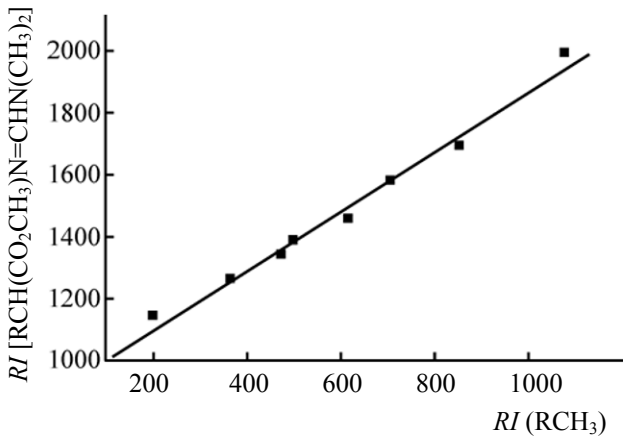


Fig. 2. Linear relation between retention indices of N-DMAM-Me derivatives of amino acids and those of the derivatives containing methyl group instead of the $–CH(CO_2CH_3)–N=CHN(CH_3)_2$ fragment.

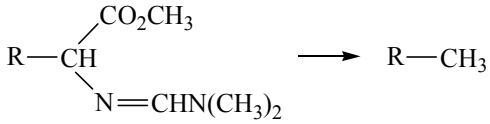
different (typically of the isomers with different number of branching in the butyl group).

Major signals in the mass spectra of the N-DMAM-Me derivatives of the studied amino acids were assigned to the ions corresponding to dimethylamino group $[NMe_2]^+$ (m/z = 44, I_{rel} = 50–100%), elimination of the methoxycarbonyl group $[M – CO_2CH_3] = [M – 59]$, the $[M – R]$ fragments (R being the α-substituent of the amino acid) (m/z = 143), and, in certain cases, $[M – NMe_2]^+$. In the presence of OH group in the R fragment (serine and threonine), the signals of the $[M – H_2O]^{+\cdot}$ ions and the corresponding secondary ions became the characteristic ones. The arginine derivative (judging from the *RI* value of 1842±1, the bis-N-DMAM-Me derivative was formed, via the amino group and the guanidine fragment) revealed the hardly interpreted mass spectrum; the lysine behavior was similar.

The behavior of the amino acids containing secondary amino groups or the NH groups in aromatic systems (proline and tryptophane) was in agreement with the known chemical properties of DMF dimethyl acetal [10]. Formation of the N-DMAM products was impossible for those substrates, they were converted into the *N*-formyl derivatives. For example, the only product in the case of proline (*M* 115) was a compound with *M* = 157 (Δ*M* = 42), corresponding to methylation of the carboxyl group (Δ*M* = 14) and *N*-formylation (Δ*M* = 28). In the case of tryptophane, two products were detected: the N-DMAM-Me derivative and the secondary product of its *N*-formylation (Table 2).

Since DMF dialkyl acetals could react with phenols and thiols as well, it was not surprising to detect two components in the products of DMF dimethyl acetal reaction with tyrosine, the first one corresponding to the normal N-DMAM-Me derivative (*M* = 250), and the second being formed via additional methylation of the phenolic hydroxyl group (*M* = 264). Signals of the molecular ions were absent in the both derivatives mass spectra, but the $[M – CO_2CH_3]$ signals at m/z = 191 and 205, respectively, were reliably detected (Table 2).

The combined use of mass spectra and gas chromatography retention indexes for identification of the N-DMAM derivatives of the amino acids opens unique possibilities for chromato–mass spectral data interpretation. Let us consider a hypothetical substitution of the $–CH(CO_2CH_3)–N=CH–N(CH_3)_2$ fragment with a simpler moiety (methyl group) taking advantage of the approach described in [17].



For convenience of the further analysis, we will discuss the parameters of the linear regression given below rather than the average Δ*RI* value for that reaction.

$$RI\,[R–CH(CO_2CH_3)–N=CH–N(CH_3)_2] = a\,RI\,[R–CH_3] + b, \quad (1)$$

$a = 0.96\pm0.04$, $b = 906\pm27$, $r = 0.992$, $S_0 = 32$, $N = 10$;

$$RI\,[R–CH_3] = a\,RI\,[R–CH(CO_2CH_3)–N=CH–N(CH_3)_2] + b, \quad (2)$$

$a = 1.03\pm0.04$, $b = –(921\pm67)$, $r = 0.992$, $S_0 = 33$, $N = 10$.

The generalized standard deviation S_0 value reflects the accuracy of the result; it should be of ±32–33 index units on the average. The plot of the linear regression (1) is given in Fig. 2.

Equations (1) and (2) formally describe the mutually inverse relations. The first one should be used to estimate the *RI* values of the N-DMAM derivatives from the *RI* data for their simple analogs RCH_3. On the contrary, the second one allows estimation of *RI* values of such simple analogs from the data for the N-DMAM derivatives of amino acids. Combined application of the equations will allow estimation of *RI* and verification of the experimental data. Let us give the examples.

Example 1. Estimation of retention indices of the N-DMAM derivatives of cycteine $HSCH_2CH(NH_2)$

CO_2H. Since the reaction with compound **I** allowed methylation of cycteine at the SH group, ethanethiol ($HSCH_2CH_3$, *RI* = 502±8) and methyl ethyl sulfide ($CH_3SCH_2CH_3$, *RI* = 605±10) should be taken as the amino acid structural analogs. Using the Eq. (1), the following estimations could be made.

$$(0.96\pm0.04)(502\pm8) + (906\pm27) \approx 1388\pm35$$
(Cys-N-DMAM-Me),

$$(0.96\pm0.04)(605\pm10) + (906\pm27) \approx 1487\pm37$$
(Cys-N-DMAM-Me_2).

The accuracy of the results was not very high, but simplicity of the method makes it attractive for preliminary estimation of the retention indices.

Example 2. The molecular ion signal was not detected in the mass spectrum of the lysine derivative with *RI* = 1961±2 (Table 2), and the mass spectrum was not informative enough to confirm the derivative structure. Let us resolve the issue using the additional chromatography data.

Lysine [$H_2N(CH_2)_4CH(NH_2)CO_2H$] interaction with DMF dimethyl acetal could result in formation of the bis-N-DMAM-Me derivative $(CH_3)_2N–CH=N–(CH_2)_4CH(CO_2CH_3)–N=CH–N(CH_3)_2$. In order to verify that, let us first estimate the retention index for its structural analog taking advantage of Eq. (2) (the errors were omitted for clarity): *RI* [$(CH_3)_2N–CH=N–(CH_2)_4CH_3$] = 1.03·1961 – 921 ≈ 1099. Then, using the ΔRI increment for the $(CH_3)_2N–CH=N–(CH_2)_4CH_3 \rightarrow H_2N(CH_2)_4CH_3$ conversion, the retention index for 1-pentanamine could be estimated, *RI* = 1099 – 396 ≈ 703. The reference *RI* value for 1-pentanamine was of 717±9, and the results agreement unambiguously confirmed the derivative structure.

The presented examples confirmed the high value of gas chromatography retention indices for interpretation of the chromato–mass spectroscopy results, including those obtained during analysis of derivatives of amino acids prepared via their modification with dimethylformamide dimethyl acetal.

EXPERIMENTAL

Dimethylformamide dimethyl acetal (Aldrich) with the major component content of 93% was used. The derivatives of the amino acids (pure grade, 1–3 mg) were prepared via the interaction with 50 μL of DMF dimethyl acetal in sealed glass ampoule with length of 40–50 mm and inner diameter of 2 mm upon heating at 80–100°C in the air thermostat of the chromatograph during 1–2 h. The sealing was necessary to prevent losses of volatile DMF dimethyl acetal (bp 102–108°C [5]). The reaction course was visually monitored by the amino acid dissolution. The so prepared mixtures were directly used for the gas chromatography analysis; the chromato–mass spectrometry analysis was performed using the mixtures diluted with dichloromethane in the 1:500 ratio.

Chromatographic analysis of the reaction mixtures was performed using a Khromatek-Kristall 5000.2 chromatograph equipped with a flame ionization detector and a BPX-1 column (standard nonpolar polydimethylsiloxane phase) with the length of 10 m and the inner diameter of 0.53 mm (the stationary phase film thickness was of 2.65 μm). The analysis conditions were as follows: temperature program of 70 to 250°C at 5 deg/min, 5.1 mL/min of nitrogen as carrier gas, splitting 6.0 : 1, evaporator temperature 250°C, specimen volume 0.2–0.3 μL.

Chromato–mass spectrometry analysis was performed using a Shimadzu 2010 Plus device in the EI ionization mode, the interface and the ion source temperature was of 200°C. An RTX-5 MS column with length of 30 m and inner diameter of 0.32 mm (the stationary phase film thickness was of 0.25 μm). The analysis conditions were as follows: temperature program of 70 to 200°C at 5 deg/min, 1.83 mL/min of helium as carrier gas, splitting 11.7 : 1, evaporator temperature 200°C, specimen volume 0.2–0.3 μL.

The linear-logarithmic retention indices of the reaction products were determined using a solution of a mixture of $C_9–C_{17}$ *n*-alkanes with the odd number of the carbon atoms in *n*-hexane as internal reference.

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