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Note

Analytical methods for the identification of short-chain carboxylic acids isolated from arthropods

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Short-chain saturated and unsaturated carboxylic acids (C_1–C_6) are frequently found as components of arthropod defensive secretions[1]. These acids and their distribution are illustrated in Table I.

TABLE I

THE SHORT-CHAIN (C_1–C_6) ACIDS FOUND IN ARTHROPOD DEFENSIVE SECRETIONS AND THEIR DISTRIBUTION

Acid	*Order*	*Family*
Formic	Hymenoptera	Formicidae
	Lepidoptera	Notodontidae
	Coleoptera	Carabidae
Acetic	Peripalpida	Thelyphonidae
	Coleoptera	Carabidae
	Hemiptera	Coreidae
n-Butyric	Coleoptera	Carabidae
β-Hydroxy-*n*-butyric	Lepidoptera	Papilionidae
Isobutyric	Lepidoptera	Papilionidae
	Coleoptera	Carabidae
	Hemiptera	Reduviidae
2-Methylbutyric	Lepidoptera	Papilionidae
n-Valeric	Coleoptera	Carabidae
Isovaleric	Coleoptera	Carabidae, Staphylinidae
n-Caproic (hexanoic)	Coleoptera	Carabidae
	Hemiptera	Coreidae
	Orthoptera	Blattidae
Methacrylic	Coleoptera	Carabidae
Ethacrylic	Coleoptera	Carabidae
Crotonic (*trans*-2-butenoic)	Coleoptera	Carabidae
Isocrotonic (*cis*-2-butenoic)	Coleoptera	Carabidae
Tiglic (2-methyl-*trans*-2-butenoic)	Coleoptera	Carabidae
Angelic (2-methyl-*cis*-2-butenoic)	Coleoptera	Carabidae

The defensive secretions of Hemiptera and Coleoptera have been the subject of recent reviews[2,3] while those from Lepidoptera were reviewed by Pavan and Valcurone-Dazzini[4] in 1976. Comparative studies on the defensive systems of 132 species of carabid beetles, belonging to 60 genera, indigenous to Japan[5], the first account of isovaleric acid in staphylinid secretions[6], and the scent composition of some East African pentatomids and coreids have been reported[7] since the preparation of these reviews.

The identification of these arthropod acids has been rather tenuous especially where more than one is produced by a single species. This paper presents gas–liquid chromatographic (GLC), high-pressure liquid chromatographic (HPLC) and thin-layer chromatographic (TLC) retention data together with mass spectral data which allow the unambiguous identification of these arthropod acids as their *p*-bromophenacyl derivatives.

MATERIALS AND METHODS

All acids were obtained from commercial sources except angelic[8] and isocrotonic[9] acid which were prepared according to literature procedures, and ethacrylic acid which was synthesised as follows. To a well stirred suspension of 75 mmole silver(I) oxide in 50 ml of water–ethanol (43:7), were added 52 mmole of 2-methylenebutanal[10] followed, over 1 h, by 20 ml of 12 *N* aqueous sodium hydroxide. The reaction mixture was stirred overnight at room temperature, filtered and the filter cake washed with warm water. The filtrate was acidified with 1 *N* sulphuric acid and extracted several times with diethyl ether. The combined ether extracts were washed with water, dried over magnesium sulphate and evaporated to a pungent oil which was distilled to yield 2.3 g of ethacrylic acid, b.p. 80–82°/13 mm (ref. 11: b.p. 81–83°/13 mm); the ^{1}H-NMR and IR spectra of the product confirmed its identity.

The *p*-bromophenacyl esters of the acids were prepared by the method of Gabriel[12]. To 1 mmole of the acid in 1 ml of 1 *N* sodium hydroxide solution was added 1 mmole 2,4′-dibromoacetophenone in 3 ml of ethanol. This solution was refluxed for 30 min, the solvent removed on the rotary evaporator at 40° and the residue dissolved in 10 ml of ether. The ethereal solution was washed with water (3 × 3 ml), dried over magnesium sulphate and the solvent evaporated. The product was recrystallized from ethanol.

GLC analyses were performed on a Perkin-Elmer Model 990 gas chromatograph fitted with flame ionization detectors. The columns used were 6 ft. × 0.125 in. I.D. stainless steel containing (a) 3% OV-225 on Chromosorb W HP (80–100 mesh) and (b) 5% OV-1 on Chromosorb W HP (80–100 mesh). The columns were operated at 190° and 200°, respectively, with a helium flow-rate of 40 ml/min. GLC–mass spectrometry was carried out on a Perkin-Elmer 990/Hitachi RMS 4 combined unit using column (a) and an operating voltage of 70 eV.

HPLC analyses were performed on a Perkin-Elmer Series 2 liquid chromatograph using a 25 × 0.26 cm Silica A/10 column, and a Perkin-Elmer LC-55 spectrometric detector at 256 nm. Various chloroform–hexane mixtures and gradients were used for elution, at a flow-rate of 1.5 ml/min.

TLC analyses were carried out on 20 × 20 cm glass plates coated with a 0.25 mm layer of Mallinckrodt Silicar 7GF, activated at 100° for 1 h. The TLC

elution solvents were (a) benzene, (b) benzene–chloroform (1:1) and (c) *n*-hexane–ethyl acetate (9:1). Detection of the spots was achieved by either observing the plates under UV light or by spraying with a 2% acidic solution of 2,4-dinitrophenylhydrazine.

All solvents used were distilled in glass, and obtained from Caledon Laboratories, Georgetown, Ontario, Canada. They were used without further purification.

RESULTS AND DISCUSSION

As has been noted by several authors the separation and identification of several of these acids as free acids or esters is not always possible by GLC[5,13,14] or TLC[13] and analysis by HPLC has been limited by the detection capabilities of the instrumentation. We have selected, as the most suitable derivative for separation and identification of these acids, the *p*-bromophenacyl ester since it is easily prepared, has good chromatographic properties and a simple diagnostic mass spectrum.

Tables II, III and IV give GLC, HPLC and TLC retention data and mass spectral data for the *p*-bromophenacyl derivatives of the short-chain acids from arthropod defensive secretions.

TABLE II

GLC RETENTION DATA FOR THE *p*-BROMOPHENACYL DERIVATIVES OF THE SHORT-CHAIN (C_1–C_6) ACIDS FOUND IN ARTHROPOD DEFENSIVE SECRETIONS

p-Bromophenacyl ester of	*Retention data*				*Mass ion*	
	3% OV-225 at 190°		*5% OV-1 at 200°*		*m/e*	*Relative abundance*
	R_t (sec) *	*R.I.* **	*R_t (sec)* *	*R.I.* **		
Formic acid	200[a]	1.29	104	0.91	242/244	3/3
Acetic acid	205[a]	1.32	128	1.12	256/258	5/5
n-Butyric acid	335[c]	2.16	224	1.96	284/286	8/8
β-Hydroxy-*n*-butyric acid	1236	8.03	376	3.29	282/284***	3/3
Isobutyric acid	259	1.67	194[c]	1.70	284/286	6/6
2-Methylbutyric acid	337[e]	2.19	264[d]	2.32	298/300	5/5
n-Valeric acid	438[b]	2.83	311	2.73	298/300	5/5
Isovaleric acid	360	2.32	270[d]	2.36	298/300	7/7
n-Caproic acid	616	4.00	432	3.79	310/312	4/4
Methacrylic acid	314	2.04	206[c]	1.81	282/284	5/5
Ethacrylic acid	409	2.66	274[d]	2.40	296/298	8/8
Crotonic acid	487	3.16	255	2.23	282/284	8/7
Isocrotonic acid	392	2.55	236	2.07	282/284	4/4
Tiglic acid	587	3.81	343	3.00	296/298	11/10
Angelic acid	440[b]	2.86	294	2.58	296/298	8/8

* Retention times followed by the same letter indicate that mixtures of these compounds were not separated.

** The retention index (R.I.) is relative to 2,4′-dibromoacetophenone which has a retention time of 154 sec on 3% OV-225 at 190°, and 114 sec on 5% OV-1 at 200°, at a helium flow-rate 40 ml/min.

*** No mass ion pair was observed for the hydroxybutyric acid, these ions represent ($M^+ - 18$)

TABLE III

HPLC RETENTION DATA FOR THE *p*-BROMOPHENACYL DERIVATIVES OF THE SHORT-CHAIN (C_1–C_6) ACIDS FOUND IN ARTHROPOD DEFENSIVE SECRETIONS

All analyses were performed on a Silica A/10 column, eluting at a rate of 1.5 ml/min with (A) 25% chloroform in hexane, (B) 20% chloroform in hexane, (C) 15% chloroform in hexane, (D) 10% chloroform in hexane, (E) 5% chloroform in hexane, (F) 10–25% gradient of chloroform in hexane at 2%/min, (G) 10–20% gradient of chloroform in hexane at 1%/min.

p-Bromophenacyl ester of	*Retention time (sec)* *						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Formic acid	285	480	—	—	—	—	—
Acetic acid	256	370	—	—	—	—	—
n-Butyric acid	132	176	256	—	—	406	428
β-Hydroxy-*n*-butyric acid	83[g]	—	—	—	—	—	—
Isobutyric acid	110	156[c]	219	—	—	360[f]	367
2-Methylbutyric acid	80[g]	121[h]	—	303[e]	541	—	—
n-Valeric acid	100[a]	150[c]	—	395	694	369[f]	390
Isovaleric acid	97[a]	145[c]	—	372[d]	757	380	414
n-Caproic acid	93[a]	124[h]	—	411	—	—	—
Methacrylic acid	107[a]	—	—	374[d]	—	—	—
Ethacrylic acid	93[a]	—	—	315[e]	—	—	—
Crotonic acid	167	—	355	—	—	—	—
Isocrotonic acid	121[b]	—	250	—	—	—	—
Tiglic acid	126[b]	—	—	—	—	—	—
Angelic acid	90[a]	—	—	315[e]	—	—	—

* Retention times followed by the same letter indicate that mixtures of these compounds were not resolved.

TABLE IV

TLC RETENTION DATA FOR THE *p*-BROMOPHENACYL DERIVATIVES OF THE SHORT-CHAIN (C_1–C_6) ACIDS FOUND IN ARTHROPOD DEFENSIVE SECRETIONS

p-Bromophenacyl ester of	*Benzene*		*Benzene–chloroform (1:1)*		*Hexane–ethyl acetate (9:1)*	
	$R_F \times 100$	$R_s \times 100$*	$R_F \times 100$	$R_s \times 100$	$R_F \times 100$	$R_s \times 100$
Formic acid	23.0	30.1	36.5	46.3	17.0	31.6
Acetic acid	15.5	20.3	30.5	38.7	19.0	35.3
n-Butyric acid	38.0	49.7	49.5	62.8	37.0	68.8
β-Hydroxy-*n*-butyric acid	0.6	0.8	2.5	3.4	1.0	2.2
Isobutyric acid	40.0	52.3	49.5	62.8	40.0	74.3
2-Methylbutyric acid	NT**	NT	NT	NT	NT	NT
n-Valeric acid	44.5	58.2	56.0	71.1	42.0	78.1
Isovaleric acid	45.5	59.5	55.0	69.8	42.0	78.1
n-Caproic acid	51.0	66.7	61.0	77.4	46.5	86.4
Methacrylic acid	42.5	53.4	49.5	66.9	33.5	74.4
Ethacrylic acid	52.5	67.1	58.0	78.4	40.5	90.0
Crotonic acid	30.5	39.0	38.0	51.4	23.0	51.1
Isocrotonic acid	43.5	55.6	43.5	58.8	28.5	63.3
Tiglic acid	55.5	72.5	65.5	83.1	34.5	64.1
Angelic acid	55.0	70.3	54.5	73.6	39.5	87.8

* The retention index R_s is calculated relative to the R_F value of 2,4′-dibromoacetophenone.
** NT = not tested.

The method of preparation used was that of Gabriel[12] in which the sodium salts of the acids were refluxed with 2,4'-dibromoacetophenone in ethanol. An alternative method, recently favoured by several workers, involves the use of a crown ether as a catalyst for the reaction of an alkali metal salt of the acid with the esterification agent, in acetonitrile[15–17]. Less than 1 μg of the derivative was detected on TLC while nanogram amounts can be detected by GLC and HPLC.

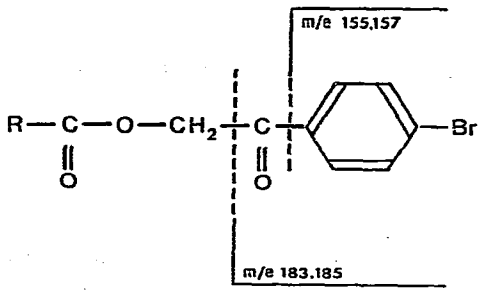

Fig. 1. Generalized scheme for the fragmentation of *p*-bromophenacyl esters of carboxylic acids.

The mass spectra of the derivatives are very simple exhibiting usually only two intense doublets at *m/e* 155/157 and 183/185 resulting from cleavage on either side of the acetophenone carbonyl group (Fig. 1) and a mass ion is cluster typical of monobromo compounds.

In the case of β-hydroxybutyric acid no mass ion is observed, the highest upscale ion being at $M^+ - 18$ arising from loss of water.

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