2,2-Dialkylacetic Acids – A New Class of Naturally Occurring Lipid Constituents

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The uropygial gland waxes of the South American red-legged Seriema (*Cariama cristata* (L., 1766)) were found to be composed of unbranched alcohols and 2,2′-dialkyl-substituted acetic acids which so far have not been found in skin lipids. When used as a chemosystematic character, the occurrence of this lipid class separates the order Cariamiformes (Seriemas) from all other avian orders hitherto investigated, especially from the Gruiformes (cranes and rails) to which they have been tentatively attributed in the past.

From the GC retention time data now available for a series of 2-alkyl-substituted fatty acid methyl esters relative retention time indices for other compounds may be predicted.

Introduction

Uropygial gland lipids are a rich source of unusual fatty acids and alcohols many of which have not been found in any other natural material (Jacob, 1978; Jacob and Ziswiler, 1982). Their structures may vary with regard to chain length, kind, localization and degree of substitution, and similar structures have been detected in closely related species, whereas qualitatively significant differences were found between higher taxa. Data on the composition of uropygial gland lipids from several hundred birds resulted in a chemosystematic approach of the class Aves which in many cases supported a link between various orders (*e.g.* Phoenicopteriformes/Anseriformes; Galliformes/Tinamiformes; Cathartiformes/Ciconiiformes). The systematic position of the Seriemas combining two South American genera (Cariama, Chunga) consisting of one species each is still under discussion, although most ornithologists favour a close relationship of the Cariamiformes to the Gruiformes (cranes and rails). The preen lipids of the latter have been analyzed, and fatty acids were found possessing a methyl branch at every fourth carbon atom (preferentially 2,6,10- and 4,8,12-trimethyl-substituted acids) (Jacob and Poltz, 1975; Jacob *et al.,* 1979). Since an uropygial gland of the Seriema (*Cariama cristata* (L., 1766)) became available to us, it appeared tempting to compare the lipids with the cranian ones. In this paper evidence is presented that both lipids differ significantly from each other. 2,2′-Dialkylacetic acids predominating in the uropygial gland lipids of the Seriema have not yet been found in any other avian organism.

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Materials and Methods

The uropygial gland was excised from a naturally died Seriema (zoo animal) and extracted with 60 ml chloroform/acetone (2:1; v/v). After the addition of 20 ml water, the lower layer, containing all lipid material, was evaporated to dryness. Thin-layer chromatography indicated that the residue consisted almost exclusively of monoester waxes. Lipids were purified on silica gel (Woelm, 14.5% water content) by elution with chloroform. The crude waxes (233 mg) were transesterified with 5% methanolic HCl to give fatty acid methyl esters and alcohols. The latter were oxidized by treatment with CrO_3/acetic acid in cyclohexane for 12 h (Jacob and Zeman, 1970) resulting in the corresponding fatty acids which then were esterified as above.

The GC separation of both fractions was carried out with 25 m×0.3 mm CPsil 5-coated glass capillaries isothermically at 220 °C column, injection port and detector temperature using a Delsi DI

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700 instrument adapted to an electronic integrator system Shimadzu CR 3A. Equivalent chain lengths (ECL values) were obtained in a semi-logarithmic plot of the retention times against chain length with reference standards of unbranched fatty acid methyl esters (n-C_{10}–C_{28}).

A reference standard of 2-octyl-dodecanoic acid was prepared by CrO_3 oxidation of 2-octyl-dodecanol which had kindly been supplied by Dr. U. Hoppe, Beiersdorf AG, Hamburg.

GC/MS was carried out with a Varian MAT 112S instrument using the above GC equipment and conditions. Mass spectra were recorded at 70 eV and 200 °C ion source temperature. Alternatively, a NERMAG R-10-10 quadrupole instrument operating at 70 eV has been used.

Results

After methanolysis of the waxes, the GC data indicated the presence of at least 3 homologous series of fatty acid methyl esters with ECL increments of 0.00, 0.10–0.15 and 0.70–0.75, respectively. With the exception of the compounds No. **18** and **19** of Table I (ECL values 16.00 and 18.00) which were identified as methyl palmitate and stearate, the presence of the mass fragment (M–59) (elimination of O=C–OCH_3) and the base peak m/z 87 gave evidence for a substitution at C-atom 2. The occurrence of even-numbered fragments at m/z 158, 186, 214 *etc.* which may be interpreted as McLafferty rearrangement ions according to Fig. 1 suggested the presence of long-

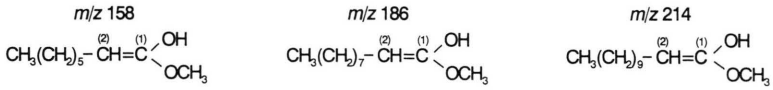

Fig. 1. McLafferty rearrangement ions m/z 158, 186 and 214.

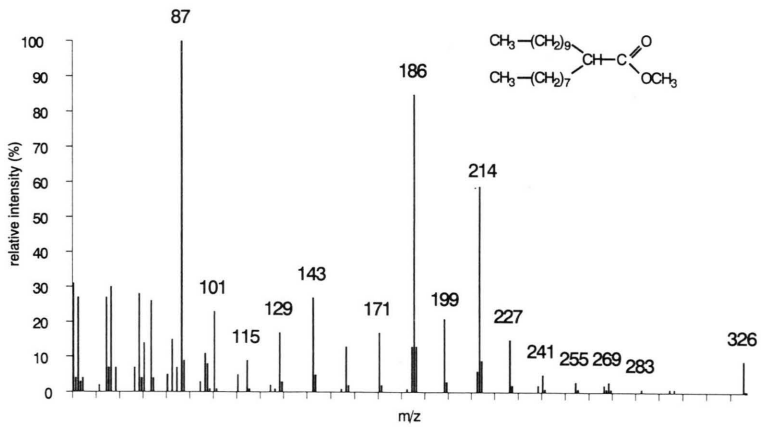

Fig. 2. Mass spectrum of a synthetic sample of methyl 2-octyldodecanoate (identical with that of compound No. **4**).

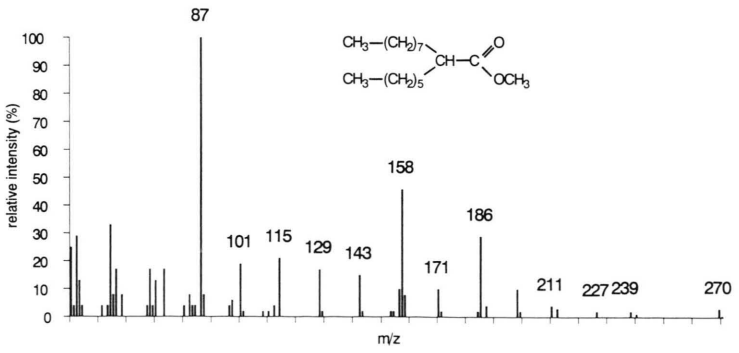

Fig. 3. Mass spectrum of compound No. **1** identified as methyl 2-hexyl-decanoate.

chain substituents at this position. This was confirmed by the mass spectrum of a synthetically prepared sample of methyl 2-octyldodecanoate which was identical with that of compound No. **4** (Fig. 2). The mass spectrum of compound No. **1,** another homologue of this series, is presented in Fig. 3 and was identified as methyl 2-hexyldecanoate.

The quantitative composition of the fatty acids from the Seriema wax along with their ECL values and the mass spectral key fragments are listed in Table I. Apart from acids with unbranched substituents at C-2, others with at least one additional branch in this chain were detected (ECL values: 17.10, 19.00, 19.05, 21.00, 21.15, 23.00, 25.00), though the position of this additional substituent could not be located.

Table I. Composition of the wax acids from the uropygial gland secretion of the Seriema as detected by GC of their methyl esters.

No.	R_1 \ CH–C / R_2 // O \ OCH_3		ECL*	Key ion	%
	R_1	R_2			
1	*n*-C_6	*n*-C_8	14.75	158, *186*	0.2
2	*n*-C_8	*n*-C_8	16.65	186	1.3
3	*n*-C_8	*br*-C_9**	17.10	*186*, 200	0.3
4	*n*-C_8	*n*-C_{10}	18.70	186, 214	12.0
5	*n*-C_8	*br*-C_{11}**	19.00	*186*, 228	0.7
6	*br*-C_9**	*n*-C_{10}	19.05	*214*, 200	1.1
7	*n*-C_9	*n*-C_{10}	19.70	214, 200	0.3
8	*n*-$C_{10(8)}$	*n*-$C_{10(12)}$***	20.70	*214*, 186, 242	34.1
9	*n*-C_{10}	*br*-C_{11}	21.00	*214*, 228	1.9
10	*br*-C_9	*n*-C_{12}	21.15	*242*, 214	0.7
11	*n*-$C_{10(9)}$	*n*-$C_{11(12)}$***	21.70	*214*, 200, 228, 242	0.4
12	*n*-$C_{10(8)}$	*n*-$C_{12(14)}$***	22.75	*214*, *242*, 186, 270	28.5
13	n–C_{10}	*br*-C_{13}	23.00	*214*, 256	1.7
14	*n*-C_{10}	*n*-C_{13}	23.75	*214*, 256	0.2
15	*n*-$C_{10(12)}$	*n*-$C_{14(12)}$***	24.75	*214*, *270*, 242	10.5
16	*n*-C_{12}	*br*-C_{13}	25.00	*242*, 256	0.4
17	*n*-$C_{12(10)}$	*n*-$C_{14(16)}$***	26.75	214, 242, 270, 298	2.9
Unbranched					
18	*n*-C_{14}	H	16.00	74	0.3
19	*n*-C_{16}	H	18.00	74	0.2
Unidentified					2.3

* ECL, equivalent chain length related to *e.g.* methyl palmitate (= 16.00).
** Branched substituent, additional branching position not located.
*** Compounds in brackets are co-eluting minor constituents.

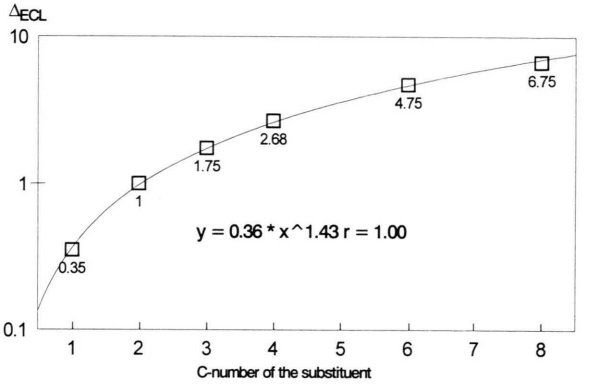

Fig. 4. ECL increments for various 2-alkyl-substituted fatty acid methyl esters (semi-logarithmic plot).

The GC data on 2-alkyl-substituted fatty acid methyl esters so far available allow to predict ECL values for other isomers of this type on the base of Fig. 4 in which increments are presented which have been measured for 2-methyl-, 2-ethyl-, 2-propyl- and 2-butyl-substituted esters (Jacob, 1975). Extrapolation to higher substituents resulted in increments which were actually found for 2-hexyl- and 2-octyl-substituted fatty acid methyl esters as found in the Seriema wax.

The quantitative composition of the wax alcohols consisting of unbranched and minor amounts of 4-methyl-alkanols is presented in Table II.

Table II. Composition of the wax alcohols from the uropygial gland secretion of the Seriema as determined by GC of the corresponding fatty acid methyl esters after oxidation with CrO_3.

No.	ECL	Compound	%
Unbranched			
1	13.00	tridecanol	0.1
2	14.00	tetradecanol	4.5
3	15.00	pentadecanol	1.6
4	16.00	hexadecanol	42.9
5	17.00	heptadecanol	1.1
6	18.00	octadecanol	26.3
7	19.00	nonadecanol	0.7
8	20.00	eicosanol	17.6
9	21.00	heneicosanol	0.1
10	22.00	docosanol	1.9
Branched			
11	16.50	4-methylhexadecanol	0.8
12	18.50	4-methyloctadecanol	0.3
13	20.50	4-methyleicosanol	0.3
Unidentified			1.8

Discussion

The uropygial gland waxes of the South American red-legged Seriema (*Cariama cristata* (L., 1766)) consists predominantly of unbranched alkanols and dialkylacetic acids the latter of which have not yet been found in the many bird waxes so far analyzed. Hence, from the chemosystematic viewpoint an exceptional systematic position has to be attributed to this species. The close relationship to the cranes which may be deduced from most of the widely accepted, though still disputed avian systematics (Wetmore, 1960; Wolters, 1975–1982; Sibley and Ahlquist, 1970) is not confirmed by this structure so that *Cariama cristata* remains isolated unless biochemically related structures are found in other avian families or orders.

The unusual structure of these lipids again raises the question on the biological significance of uropygial gland secretions other than making the plumage waterproof. Various preen gland constituents have been found to possess bactericidal and/or fungicidal properties such as 3,7-dimethyloctanol-1 which has recently been detected as alcoholic constituent of the uropygial gland wax from the gannet *(Sula bassana)* (Jacob *et al.,* 1993). From some fatty acids, structurally related or identical with those occurring in the Seriema wax, which have previously been identified in the uropygial gland secretions of owls (Jacob and Poltz, 1974; Jacob and Hoerschelmann, 1984), especially 2-ethyl-, 2-butyl- and 2-hexyldecanoic acid and their methyl esters, larvicidal activities against the mosquito *Culex pipiens quinquefasciatus* (Say.) have been reported (Hwang *et al.,* 1974, 1976). Presently, some wax constituents from the Seriema are tested against a broader variety of microorganisms the results of which will be published elsewhere.

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