Published in
International Journal of Invertebrate Reproduction and Development 8, 1-13, 1985
which should be used for any reference to this work

A new class of apolar ecdysteroid conjugates: esters of 20-hydroxy-ecdysone with long-chain fatty acids in ticks

Peter A. Diehl [1,*], Jean-Louis Connat [1], Jean P. Girault [2] and René Lafont [3]

[1] *Institut de Zoologie, Université, Chantemerle 22, CH-2000 Neuchâtel, Suisse;* [2] *Ecole Normale Supérieure, Laboratoire de Chimie, CNRS L.A.32, 24 rue Lhomond, 75231 Paris Cedex 05; and* [3] *Ecole Normale Supérieure, Laboratoire de Zoologie, CNRS U.A.686, 46 rue d'Ulm, 75230 Paris Cedex 05, France*

Summary

Nymphs of the argasid tick *Ornithodoros moubata* rapidly transform most of the ingested molting hormone 20-hydroxy-ecdysone (20-hydroxy-E) into a family of 4 major apolar conjugates which are hydrolyzable by carboxylic-ester hydrolase. These conjugates – new among zoo- or phytoecdysteroids – are composed of 20-hydroxy-E esterified at C22 with the common long-chain fatty acids $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, or $C_{18:2}$. The chemical structure of the purified metabolites was identified by fatty acid analysis, by CI/D mass spectrometry and by comparison with a chemically prepared 20-hydroxy-E-22-palmitate.

The significance of the apolar metabolic pathway is discussed. It may serve to inactivate molting hormones which could be ingested with blood from hosts that feed on plants containing phytoecdysteroids. In addition, the conjugates may act as a storage form of hormones for the developing embryo.

ecdysteroids; metabolism; ecdysteroid conjugates; ticks

Introduction

In insects, the metabolism of the molting hormones ecdysone (E) and 20-hydroxy-ecdysone (20-hydroxy-E) can follow several pathways: (1) hydroxylation to

* To whom correspondence should be addressed.

26-hydroxy-E or 20,26-dihydroxy-E, (2) oxidation to 26-carboxy-E or 26-carboxy-20-hydroxy-E, (3) conjugation with polar moieties (e.g. phosphates, acetophosphates, nucleotides, sulfates), (4) oxidation at C3 to 3-dehydro-ecdysteroids, (5) epimerization at C3, (6) side-chain cleavage, and (7) formation of less polar E- or 20-hydroxy-E-3-acetates [1,2]. Most of these metabolites are inactivation products and are excreted by the animal. Others, such as ecdysteroid-22-phosphates and/or nucleotides, are stored in the eggs where they may represent a source of hormone for the early developing embryo [3,4].

In the argasid tick *Ornithodoros moubata*, E or 20-hydroxy-E is metabolized (i) in a polar pathway to presumably 20,26-dihydroxy-E and further to polar ionizable metabolites of unknown chemical nature, and (ii) to a large extent to apolar conjugates [5,6]. In nymphs, both polar and apolar metabolites accumulate in the midgut and are thus likely to be inactivation products. In females, only apolar conjugates are produced after injection of E or 20-hydroxy-E. Important amounts of these conjugates are transferred to the eggs where they might act as a hormone storage form for the embryo [6].

Here we report on the isolation and chemical identification of these apolar conjugates produced after ingestion of 20-hydroxy-E by nymphs of *O. moubata*. This metabolite class – new for arthropods – is composed of conjugates of 20-hydroxy-E esterified at C22 with the common fatty acids $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, or $C_{18:2}$.

Materials and methods

Animals

Fifth stage nymphs of the argasid tick *O. moubata* (Murray, 1877; sensu Walton, 1962) were fed on defibrinated pig blood at 37°C through a Parafilm membrane. They were kept in glass tubes plugged loosely with cotton at about 30–40% relative humidity in the dark. In these conditions the nymphs molt to females 9–10 days after the meal.

Chemicals

Solvents were of analytical or chromatographical grade. The ecdysteroids ecdysone (E) and 20-hydroxy-E (structure in Fig. 1) were purchased from Simes (Italy). Tritiated E was bought from New England Nuclear (spec. act. about 50 Ci/mmol). Labelled 20-hydroxy-E was prepared by in vitro conversion of [^{3}H]E with locust Malpighian tubules followed by high performance liquid chromatography (HPLC) purification.

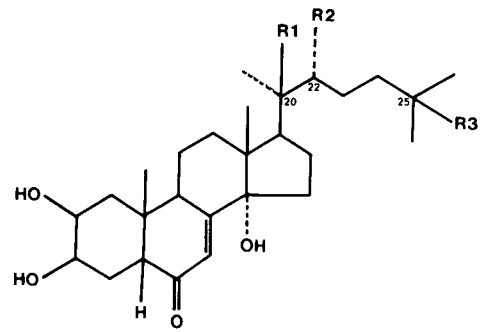
Preparation of apolar conjugates

One hundred nymphs were allowed to feed on 20 ml of blood containing 35 μg/ml of 20-hydroxy-E and about 500 000 cpm/ml of 20-[^{3}H]hydroxy-E. Hormones had been dissolved in the blood by adding them in a few μl of ethanol followed by vigorous shaking. 24 h later, the ticks were homogenized and sonicated in 100 ml methanol. After centrifugation, the pellets were re-extracted twice with 100 ml

methanol. The combined supernatants were evaporated to near dryness and then taken up in about 6 ml of methanol–water (9 : 1). After sonication and centrifugation, the supernatant was further purified by preparative HPLC.

HPLC

HPLC analyses were performed on a Perkin–Elmer Series 3 or on a Dupont chromatograph equipped with variable wavelength spectrophotometers operated at 242 nm. Preparative HPLC was achieved on a RP-18 column (Knauer, 16 × 250 mm; particle size: 10 μm) using a gradient of methanol-Tris/perchloric acid buffer (20 mM, pH 7.5) and solvent system 1: linear gradient of 90% methanol buffer to 100% methanol in 10 min at 2.5 ml/min followed by 100% methanol for 40 min at 2 ml/min at room temperature. Analytical HPLC was performed on RP-18 columns (Hibar, Merck; 4 × 250 mm, Lichrosorb RP-18, 5 or 7 μm) using solvent system 2: linear methanol buffer gradient from 30–45% methanol in 10 min, 45% for 15 min, 45–100% for 20 min followed by 100% for 20 min at 0.8 ml/min. Final purification of the apolar conjugates was achieved by HPLC on silica. Each of the three apolar fractions purified by preparative HPLC was injected separately on a Zorbax-SIL column (4.6 × 250 mm) using solvent system 3: methylene chloride–isopropanol–water (125 : 15 : 1) at 1 ml/min at room temperature. Purification of the synthetic 20-hydroxy-E-palmitates was achieved by repetitive Zorbax-SIL (sys-



	R1	R2	R3
Ecdysone	-H	-OH	-OH
20-Hydroxyecdysone	-OH	-OH	-OH
22,25-Dideoxyecdysone	-H	-H	-H

Fig. 1. Structural formulae of ecdysone (= 2β, 3β, 14α, 22R, 25-pentahydroxy-5β-cholest-7-ene-6-one), 20-hydroxy-ecdysone (= 2β, 3β, 14α, 20R, 22R, 25-hexahydroxy-5β-cholest-7-ene-6-one), and 22, 25-dideoxy-ecdysone (= 2β, 3β, 14α-trihydroxy-5β-cholest-7-ene-6-one).

tem 3) or with Hibar columns (Merck; Lichrospher 5 μm or Lichrosorb 7 μm, 4 × 250 mm) with solvent system 4 chloroform–isopropanol–water (125 : 13.5 : 0.6) or 5 (125 : 20.5 : 1). Systems 4 or 5 were also used for the separation of the 20-hydroxy-E-palmitates which had been derivatized with acetic anhydride or palmitic anhydride (see below).

Enzymatic hydrolysis of conjugates

Crude extracts or purified conjugates were dried under a stream of N_2 and then hydrolyzed with 200 μl of hog liver esterase (carboxylic-ester hydrolase, EC 3.1.1.1; Boehringer) and 1.8 ml of borate buffer (0.1 M, pH 8). After an overnight incubation at 37°C, the products were extracted with methanol and analysed by HPLC. Only little hydrolysis was observed without added enzymes.

Liquid scintillation counting

Radioactivity was monitored by liquid scintillation counting in a Kontron MR 300 counter. Samples were dissolved in Riatron scintillation cocktail (Kontron) (1 : 1.75 v/v).

Analysis of fatty acids

Fatty acid analysis was performed on pure conjugates according to Carreau and Dubacq [7]. The conjugates (20–50 μg) were dissolved in 0.5 ml methanol solution of sodium methylate (1% w/v) and heated for 5 min at 55°C. Then 0.5 ml 1 N hydrochloric acid in absolute methanol was added and the mixture again heated for 5 min at 55°C. Then 1 ml pentane and 1 ml water were added. The upper layer (pentane), containing the methyl esters of fatty acids was analysed by GLC–FID (injection of 4 μl). GLC apparatus: Girdel 300 with a Pyrex capillary column (l = 25 m, PEG 20000). Carrier gas: helium ($p = 0.7$ bar). Isothermal conditions (170°C). $C_{17:0}$ fatty acid methyl ester was added as an internal standard. The fatty acids were identified by comparison with the retention times of reference fatty acid methyl esters.

Mass spectrometry

Mass spectrometry was carried out with a Riber 10-10B apparatus (Nermag S.A., France) equipped with a direct inlet probe. Spectra were recorded using a chemical ionization/desorption (CI/D) procedure with ammonia as the reagent gas [8].

[^{1}H]NMR analysis

Proton-NMR spectra in $CDCl_3$ were obtained by Fourier transform techniques with a spectrometer type WM250 (Bruker).

Synthesis of monopalmitate esters of 20-hydroxy-E

Reference monopalmitate esters of 20-hydroxy-E were synthesized by reacting 80 mg of hormone with about 120 mg of palmitic anhydride (Grade I, Sigma) dissolved in 350 μl of dry pyridine (5h, 50°C). The products were purified by repetitive HPLC on silica and their structure determined by mass spectrometry and [^{1}H]NMR. Yield:

unreacted hormone: 28%; 20-hydroxy-E-2-palmitate: 60%; 20-hydroxy-E-22-palmitate: 2.5%; rest: small amounts of 20-hydroxy-E-3-palmitate and 20-hydroxy-E-dipalmitates.

Derivatisation of 20-hydroxy-E-22-palmitate

A mixture of synthetic and purified biological [^{3}H]20-hydroxy-E-22-palmitates was acetylated for 2, 40 or 180 min [9] or esterified for 30 or 100 min with palmitic anhydride. The reaction mixtures were thereafter analysed by silica HPLC for co-chromatography of the UV-absorbing peaks with the radioactive peaks.

Results

(1) Formation of apolar conjugates after ingestion of 20-hydroxy-E

Within 24 h of the ingestion of 35 μg/ml of 20-hydroxy-E, about 90% of the hormone was converted to three major apolar conjugate fractions 1–3 which elute on analytical or preparative RP-18 columns well after 22,25-dideoxy-E (Fig. 2).

Each of these three collected conjugate fractions was then further purified by HPLC on silica. Surprisingly, they eluted between synthetic 20-hydroxy-E-3-acetate and 20-hydroxy-E-22-acetate, behaving thus as compounds much more polar than was expected from their very apolar behaviour on RP-18 columns. Fraction 1 contained two very small peaks and one major peak which corresponded to about 15% of the apolar conjugates. Fraction 2 could be separated into two peaks (2A: 35%, 2B: 24%), and fraction 3 yielded a single peak (3: 18% of the conjugates).

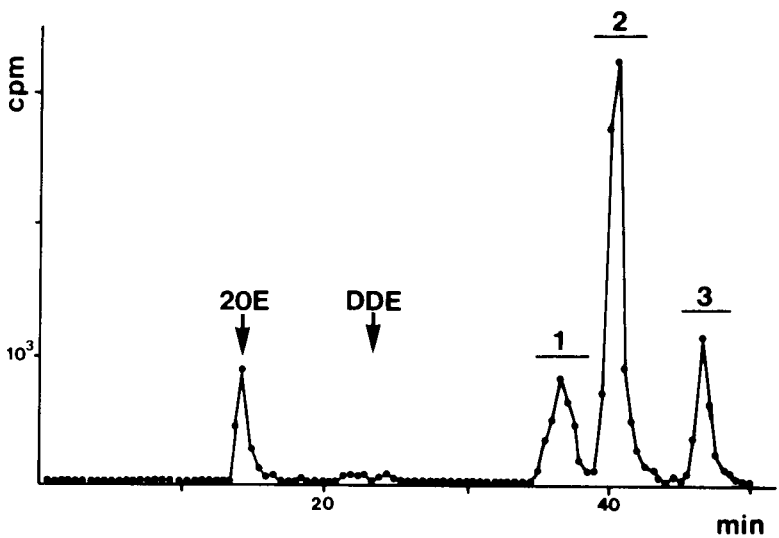


Fig. 2. Preparative HPLC (RP-18) of metabolites present in ticks 24 h after ingestion of [^{3}H]20-hydroxy-ecdysone. Retention times of 20-hydroxy-ecdysone (20E) and 22,25-dideoxy-ecdysone (DDE) are marked. The apolar conjugate fractions 1,2 and 3 were further purified by silica HPLC.

(2) Apolar conjugates are fatty acid esters

The apolar conjugates proved to be hydrolysable by carboxylic-ester hydrolase (EC 3.1.1.1) thus yielding the original 20-hydroxy-E. This ester nature was confirmed by the GLC analysis of the methylated fatty acids after chemical hydrolysis. Conjugate 1 contained linoleic acid, 2A oleic acid, 2B palmitic acid and 3 stearic acid (Table I). The apolar conjugates are thus composed of 20-hydroxy-E esterified with the common long-chain fatty acids.

Conjugation a priori is likely to occur with the most reactive secondary hydroxyl groups at C2, C3 or C22 [2]. For further chemical details we needed thus other analyses by mass spectrometry and by comparison with synthetic reference compounds.

(3) Analysis of conjugate 2B

Mass spectrometry (MS) by CI/D of conjugate 2B gave the following ions: m/z 719 ($M + H^+$), 701 ($719-H_2O$), 683 ($719-2H_2O$), 665 ($719-3H_2O$), then 463 (719–fatty acid), 445 (100%; $463-H_2O$), 427 ($463-2H_2O$), and 409 ($463-3H_2O$). Some ions were also recorded at m/z 363 (719–fatty acid–side chain) and 345 ($363-H_2O$) (Fig. 3A). These data are consistent with the presence of a monopalmityl ester of 20-hydroxy-E. Furthermore, the lack of any ion at m/z 601 (MH^+-118) (118 = fragment C22–C27 of 20-hydroxy-E, lost easily in 20-hydroxy-E, 20-hydroxy-E-2- or 20-hydroxy-E-3-acetate (Fig. 3C)) allows us to exclude the esterification

TABLE I

Molecular masses of the conjugates and the fatty acids liberated after chemical hydrolysis

Conjugate	Molecular mass	Fatty acid
1	742	$C_{18:2}$
2A	744	$C_{18:1}$
2B	718	$C_{16:0}$
3	746	$C_{18:0}$

TABLE II

Retention volume of various ecdysteroids on a silica column (Lichrospher 5 μm). Solvent system 5 at 1.2 ml/min

Compound	Retention volume (ml)
20-Hydroxy-E-2-palmitate	8.4
20-Hydroxy-E-3-palmitate	9.6
Ponasterone A	13.2
2-Deoxy-E	15.6
20-Hydroxy-E-2-acetate	20.4
20-Hydroxy-E-3-acetate	21.6
20-Hydroxy-E-22-palmitate	28.8
20-Hydroxy-E-22-acetate	34.8

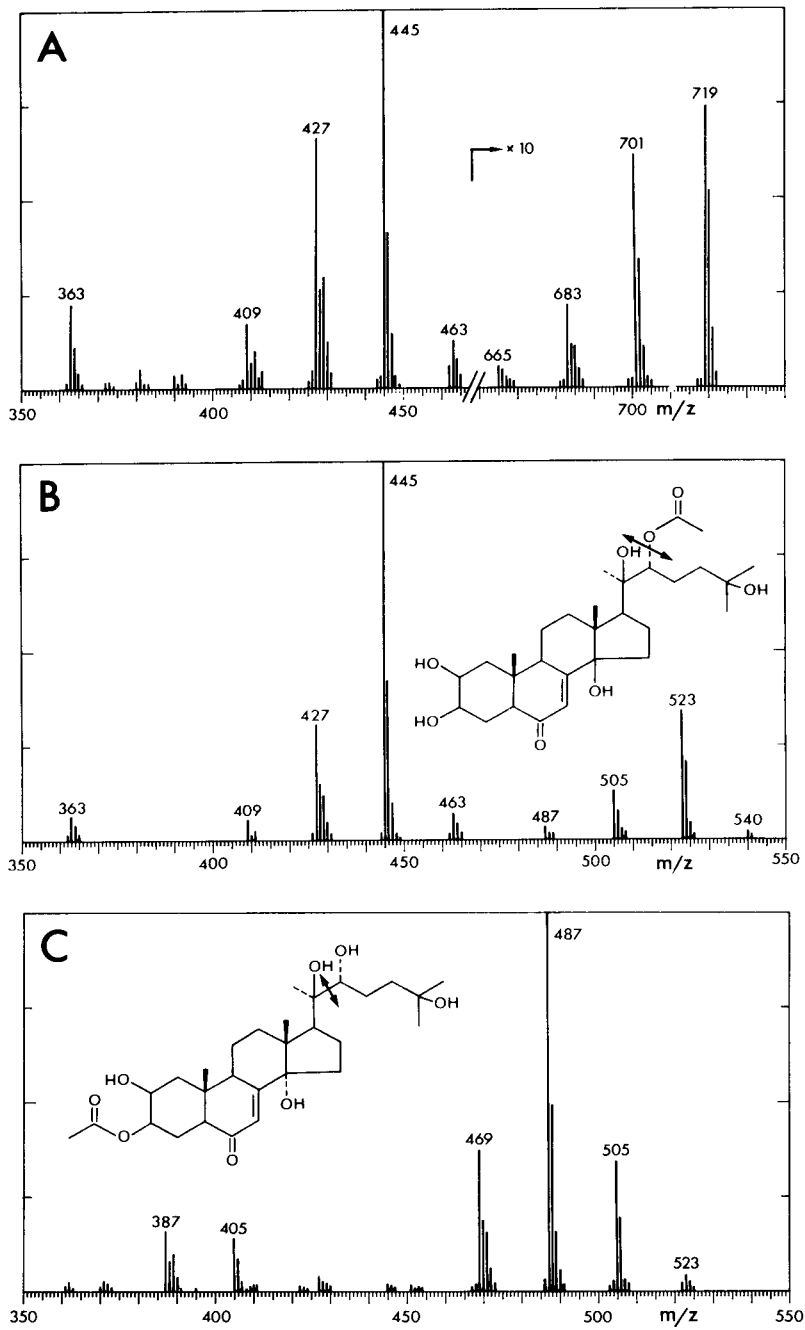


Fig. 3. Mass spectra (CI/D) of 20-hydroxy-E-22-palmitate (A), 20-hydroxy-E-22-acetate (B) and 20-hydroxy-E-3-acetate (C).

involving C2 or C3. The MS is thus very similar to that of 20-hydroxy-E-22-acetate (Fig. 3B). It can only involve C22 (or possibly the less reactive C25 position).

For further identification, we prepared synthetic 20-hydroxy-E-22-palmitate by reacting 20-hydroxy-E with palmitic anhydride. The synthesis yielded a rapidly migrating major compound (1) and a minor compound (2) which eluted on silica much later between 20-hydroxy-E-3-acetate and 20-hydroxy-E-22-acetate (Table II). The [^{1}H]NMR spectrum (250 MHz, $CDCl_3$, internal standard TMS) of compound (1) gave signals at δ 0.86 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.20 (3H, s, 21-CH_3), 1.23–1.24 (6H, 2s, 26/27-CH_3), 2.30 (2H, *t*, –O–CO–CH_2–*R*, $J \simeq 7$ Hz), 2.50 (1H, *q*, 5-H, $J_{4ax,5} = 13.5$ Hz, $J_{4eq,5} = 4.5$ Hz), 3.08 (1H, m, 9-H, $W_{1/2}$ 21 Hz), 3.44 (1H, d, 22-H, $J_{22,23a} = 11$ Hz, $J_{22,23b} \leqslant 1$ Hz), 4,10 (1H, m, 3-H_{eq}, $W_{1/2} = 8$ Hz), 5.01 (1H, m, 2-H_{ax}, $W_{1/2} = 20$ Hz, $J_{lax,2} = 11.5$ Hz, $J_{leq,2} = 4.5$ Hz), 5.84 (1H, d, 7-H, $J_{7,9} = 2.6$ Hz) and for compound (2) δ 0.84 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.23-1.19 (9H, 3s, 21/26/27-CH_3), 2.36 (2H, t, -O-CO-CH_2-R, $J \simeq 7$ Hz), 2.42 (1H, q, 5-H, $J_{4ax,5} = 13.5$ Hz, $J_{4eq,5} = 4.5$ Hz), 2.99 (1H, m, 9-H, $W_{1/2} = 21$ Hz), 3.89 (1H, m, 2-H_{ax}, $W_{1/2} = 22$ Hz), 4.04 (1H, m, 3-H_{eq}, $W_{1/2} = 8$ Hz), 4.85 (1H, d, 22-H, $J_{22\text{-}23a} = 11$ Hz, $J_{22\text{-}23b} \leqslant 1$ Hz), 5.83 (1H, d, 7-H, $J_{7,9} = 2.6$ Hz). For the 20-hydroxy-E one noted the following signals at δ 0.86 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.21 (3H, s, 21-CH_3), 1.23 and 1.24 (6H, 2s, 26/27-CH_3), 2.43 (1H, q, 5-H, $J_{5,4ax} = 14$ Hz, $J_{5,4eq} = 4.5$ Hz), 2.98 (1H, m, 9-H, $J_{9,1lax} = 11.3$ Hz, $J_{9,1leq} = 7.2$ Hz, $J_{9,7} = 2.5$ Hz, $W_{1/2} = 22$ Hz), 3.44 (1H, d, 22-H, $J_{22,23a} = 10$ Hz, $J_{22,23b} \leqslant 1$ Hz), 3.89 (1H, m, 2-H_{ax}, $J_{2,lax} = 11.7$ Hz, $J_{2,leq} = 4.7$ Hz, $J_{2,3e} = 3.3$ Hz, $W_{1/2} = 20$ Hz), 4.04 (1H, m, 3-H_{eq}, $W_{1/2} = 7$ Hz), 5.84 (1H, d, 7-H, $J_{7,9} = 2.5$ Hz).

All these proton signals are clearly assigned by their chemical shifts, width at half-height [14], coupling patterns and coupling constants, using homonuclear decoupling technics, two-dimensional COSY 45 experiments [15], and with comparison of the resolution enhanced and computer simulated spectras.

Isomer *1* shows a large downfield shift (2-H_{ax} = +1.1 ppm) of the 2-H_{ax} signal and small downfield shifts for the 3-H_{eq}, 5-H, 9-H signals (no change for the 22-H) with regard to the 20-hydroxy-E's protons. On the other hand, isomer *2* shows only a large downfield shift of the 22-H signal (= +1.4 ppm). Thus isomer *1* is assigned as the 20-hydroxy-E-2-palmitate isomer and isomer *2* as the 20-hydroxy-E-22-palmitate.

The synthetic 20-hydroxy-E-22-palmitate showed the same MS fragmentation pattern as the biological conjugate 2B. Furthermore, both synthetic 20-hydroxy-E-22-palmitate and conjugate 2B co-migrated on silica and RP-18 analytical or preparative columns. In addition, co-acetylation or co-acylation (with palmitic anhydride) of both compounds for different lengths of time yielded UV-absorbing and radiolabelled products that comigrated on silica columns. From these data we deduce that the biological conjugate 2B is 20-hydroxy-E-22-palmitate.

(4) Structure of the conjugates 1, 2A and 3

The fatty acid moieties of the conjugates 1, 2A and 3 are also attached to C22 because they show not only comparable retention times on silica but also the same MS fragmentation pattern as 20-hydroxy-E-22-palmitate: all of them gave base

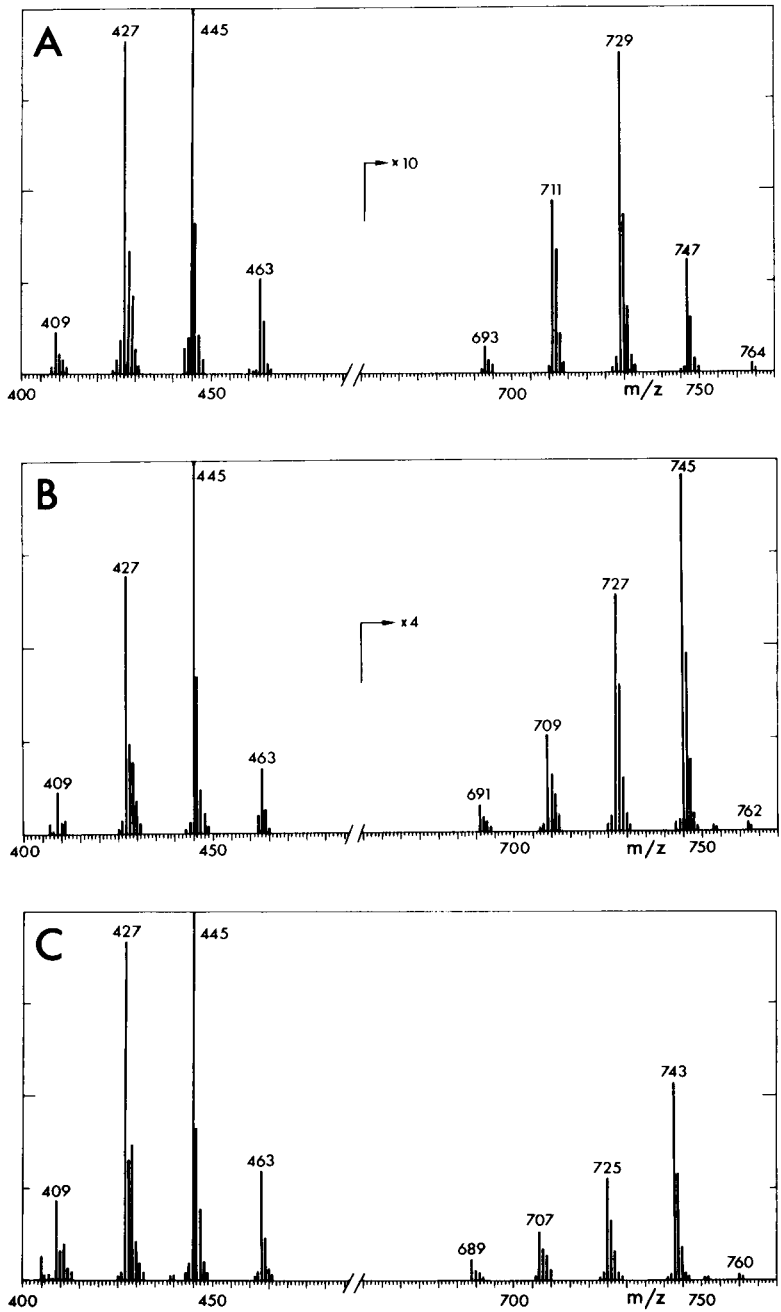


Fig. 4. Mass spectra (CI/D) of 20-hydroxy-E-22-stearate (A), 20-hydroxy-E-22-oleate (B) and 20-hydroxy-E-22-linoleate (C).

peaks at m/z 445 (Fig. 4). The differences were observed in the high masses which are in agreement with the masses of the different fatty acids identified by GLC (Table I).

Discussion

Our results show unambiguously that the ticks rapidly transformed most of the ingested hormone into four major apolar conjugates which consist of 20-hydroxy-E esterified at C22 with the common long-chain fatty acids $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, or $C_{18:2}$. These conjugates constitute a new class of metabolites among zoo- or phytoecdysteroids (cf. refs. 1, 2, 10, 11). Comparable ecdysteroid acyl esters, but with the short-chain acetic acid, occur in locusts (E- and 20-hydroxy-E-2- or-3-acetates and acetophosphates [12,13,16]) and in plants (cyasterone-22-acetate, 20-hydroxy-E-25-acetate or pinnasterol-3-acetate [10,11]). In addition, other apolar conjugates are known in plants where the ecdysteroids are esterified with cinnamic, coumaric, or benzoic acid [10,11].

It is also interesting to note recent reports on the presence of comparable apolar steroid hormone esters in vertebrates. For example, bovine adrenal mitochondria convert pregnenolone to pregnenolone arachidonate, linoleate, oleate, palmitate, or stearate [17]. Similarly, estradiol can be converted into a family of 10 fatty acid esters, where the hormone is esterified at C17 mostly with polyunsaturated fatty acids such as arachidonic acid [18].

It was rather surprising to note the great change of polarity of the conjugates depending on the HPLC column. In the reverse phase mode, the esters behave as very apolar compounds due to the presence of the long hydrophobic fatty acid tail. Thus the esters separate according to the length and the degree of unsaturation of the fatty acids ($C_{18:0}$ the least polar, $C_{16:0}$ and $C_{18:1}$ co-migrating in an intermediate position, $C_{18:2}$ the most polar). On the other hand, the conjugates migrate on silica as much more polar compounds than expected from their behaviour on RP-18 – they elute between 20-hydroxy-E-3-acetate and 20-hydroxy-E-22-acetate. This shows the great influence of the free 2- and 3-OH for the retention on silica. 20-Hydroxy-E-2- or 20-hydroxy-E-3-palmitate are much less polar. A similar elution pattern can also be noticed for the 20-hydroxy-E-2-, 20-hydroxy-E-3-, and 20-hydroxy-E-22-acetates. This chromatographic behaviour indicates that a combination of HPLC on RP-18 and silica is necessary for complete purification of the conjugates. Indeed, RP-18 columns do not resolve the $C_{16:0}$ and $C_{18:1}$ esters, which are, however, resolved on silica.

The esterification of ingested or injected ecdysteroids containing a 22-OH group is very active not only in nymphs but also in females [6]. We postulate that this conjugation might act as an inactivation mechanism directed against exogenous ecdysteroids which may be ingested with blood from herbivorous hosts. Indeed, many plants contain ecdysteroids, sometimes even in very large concentrations [11], and they may therefore be present in the host's blood. Such an inactivation mechanism seems to be necessary because *O. moubata* is sensitive to exogenous

ecdysteroids [19]. However, only large doses of 5–20 μg of the 22-OH containing E, 20-hydroxy-E, Makisterone A, or Ponasterone A ingested by a female induced supermolting and reduced fecundity. On the other hand, doses as low as 20 ng of 22, 25-dideoxy-E were sufficient to induce the same effects [19]. Because very large doses of 22-hydroxy-ecdysteroids are needed to produce a biological effect, we can reasonably deduce that the esterification mechanism at C22 is very efficient in coping with hormone concentrations of up to several μg/ml.

From preliminary in vitro studies, it appears that the midgut is especially active in hormone conjugation. The importance of the midgut barrier is underlined by the fact that, in contrast to ingestion, injection of only about 100 ng of E or 20-hydroxy-E into the hemolymph of a female is sufficient to induce supermolting and egg resorption (in preparation). It appears thus reasonable to postulate that conjugation, especially in the midgut, is an efficient inactivation mechanism for possibly ingested ecdysteroids. However, it remains to be demonstrated that (i) the ecdysteroid esters are biologically inactive, and (ii) vertebrate blood can indeed contain ecdysteroids derived from plant food, although this is very strongly suggested from experiments where orally administrated ecdysteroids influenced serum cholesterol content in rabbits [20] or caused early stimulation of protein synthesis in murine liver [21].

Interestingly, locusts produce chemically comparable ecdysteroid-acetates [12,13,16]. Acetylation is very active in the intestine, especially in the gastric caecae of *Locusta* larvae [22]. Like the tick conjugates, these locust metabolites are strongly suspected to be inactivation products.

Vitellogenic females of *O. moubata* also rapidly convert ecdysteroids into apolar esters which accumulate to a considerable extent in the developing oocytes [6]. It is therefore possible that these conjugates might serve as a hormone storage form from which free ecdysteroids could be liberated during embryogenesis. This would be comparable to the situation in locusts where E-22-phosphate in *Schistocerca* [4] or 22-adenosine monophosphate esters of E or 2-deoxy-E in *Locusta* [3] represent a hormone source for the embryo. In this context, it is also interesting to note that the human estradiol fatty acid esters are suspected to act as a slow release device from which free hormone might be liberated [23]. However, only future studies on the fate of the ecdysteroid esters during tick embryogenesis will give us an answer about their physiological role during this life period.

Formation of apolar ecdysteroid acyl esters with long-chain fatty acids are not restricted to the argasid tick *O. moubata*. We have detected these metabolites also in nymphs and females of the ixodid tick *Amblyomma hebraeum* (in preparation). In addition, apolar hydrolysable ecdysteroid fatty acid esters have also been observed in females of the ixodid *Boophilus microplus*, although their precise chemical nature has not yet been reported [24]. Furthermore, hydrolysable apolar ecdysteroid esters occur also in *Drosophila* females [25]. They co-chromatograph on RP-18 or silica with the tick conjugates (collaborative work with Dr. Dübendorfer). Other work in progress demonstrates the presence of similar ecdysteroid acyl esters in several other arthropods. These preliminary data show that this new class of ecdysteroid metabolites is not restricted to ticks. Indeed, they may have been easily overlooked in the past by the currently used ecdysteroid detection procedures due to a low cross-reac-

tivity in the RIA (about 300 times less reactive in our RIA [26]) and due to their rather good resistance to treatment with *Helix* juice. Thus more work is needed to map the distribution of these conjugates among arthropods and to precisely establish their physiological role.

Acknowledgements

We are grateful for the generous financial support by the Swiss National Science Foundation (Request No. 3.070.-0.81) and by the Emil Barrel Stiftung. We thank M. Morici for performing HPLC analyses, E. Dotson for correcting the English, and G. Darioly for typing the manuscript. Dr. J.-P. Dubacq kindly helped with the GLC analysis of the fatty acids.

References

1 Koolman, J., Ecdysone metabolism. Insect Biochem., 12 (1982) 225–250.
2 Lafont, R. and Koolman, J., Ecdysone metabolism. In J.A. Hoffmann and M. Porchet (eds.), Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, Springer-Verlag, Heidelberg, 1984, in press.
3 Sall, C., Tsoupras, G., Kappler, C., Lagueux, M., Zachary, D., Luu, B. and Hoffmann, J., Fate of maternal conjugated ecdysteroids during embryonic development in *Locusta migratoria*. J. Insect Physiol., 29 (1983) 491–507.
4 Isaac, E.R., Sweeney, F.P. and Rees H.H., Enzymic hydrolysis of ecdysteroid phosphate during embryogenesis in the desert locust (*Schistocerca gregaria*). Biochem. Soc. Trans., 11 (1983) 380–381.
5 Bouvier, J., Diehl, P.A. and Morici, M., Ecdysone metabolism in the tick *Ornithodoros moubata* (Argasidae, Ixodoidea). Rev. Suisse Zool., 89 (1982) 967–976.
6 Connat, J.L., Diehl, P.A. and Morici, M., Metabolism of ecdysteroids during the vitellogenesis of the tick *Ornithodoros moubata* (Ixodoidea, Argasidae): accumulation of apolar metabolites in the eggs. Gen. Comp. Endocrinol., 56 (1984) 100–110.
7 Carreau, J.P. and Dubacq, J.P., Adaptation of a macroscale method to the micro-scale for fatty acid methyl transesterification of biological lipid extracts. J. Chromatog., 151 (1978) 384–390.
8 Lafont, R., Beydon, P., Mauchamp, B., Somme-Martin, G., Andrianjafintrimo, M. and Krien, P., Recent progress in ecdysteroid analytical methods. In F. Sehnal, A. Zabza, J.J. Menn and B. Cymborowsky (eds.), Regulation of Insect Development and Behaviour, Technical University of Wroclaw Press, 1981, pp. 125–144.
9 Koolman, J., Reum, L. and Karlson, P., 26-Hydroxyecdysone, 20,26-dihydroxyecdysone and inokosterone detected as metabolites of ecdysone in the blowfly *Calliphora vicina* by radiotracer experiments. Hoppe-Seylers Z. Physiol. Chem., 360 (1979) 1351–1355.
10 Hetru, C. and Horn, D.H.S., Phytoecdysteroids and zooecdysteroids. In J.A. Hoffmann (ed.), Progress in Ecdysone Research, Elsevier/North-Holland Biomedical Press, Amsterdam, New York, Oxford, 1980, pp. 13–28.
11 Bergamasco, R. and Horn, D.H.S., Distribution and role of insect hormones in plants. In R.G.H. Downer and H. Laufer (eds.), Endocrinology of Insects, Alan R. Liss Inc., New York, 1983, pp. 627–654.
12 Tsoupras, G., Luu, B., Hetru, C., Muller, J.F. and Hoffmann, J.A., Conversion in vitro de 20-hydroxyecdysone en métabolites phosphorylés et acétylés par des complexes tubes digestif-tubes de Malpighi de larves de *Locusta migratoria*, C.R. Acad. Sci. (Paris), 296 (1982) 77–80.
13 Lafont, R., Blais, C., Beydon, P., Modde, J., Enderle, U. and Koolman, J., Conversion of ecdysone

and 20-hydroxyecdysone into 26-oic derivatives is a major pathway in larvae and pupae of species from three insect orders. Arch. Insect Biochem. Physiol., 1 (1983) 41–58.
14 Horn, D.H.S., The ecdysones. In M. Jacobson and D.G. Crosby (eds.), Naturally Occurring Insecticides, Marcel Dekker, New York, 1971, pp. 333–459.
15 Bax, A. and Freeman, R., Investigation of complex networks of spin-spin coupling by two dimensional NMR. J. Magn. Res., 44 (1981) 542–561.
16 Isaac, R.E., Rees, H.H. and Goodwin, T.W., Isolation of ecdysone-3-acetate as a major ecdysteroid from the developing eggs of the desert locust. Chem. Commun. (1981) 594–595.
17 Mellon-Nussbaum, S., Ponticorvo, L. and Lieberman, S., Characterization of the lipoidal derivatives of pregnenolone prepared by the incubation of the steroid with adrenal mitochondria. J. Biol. Chem., 254 (1979) 12500–12505.
18 Mellon-Nussbaum, S.H., Ponticorvo, L., Schatz, F. and Hochberg, R.B., Estradiol fatty acid esters. The isolation and identification of the lipoidal derivative of estradiol synthesized in the bovine uterus. J. Biol. Chem., 257 (1982) 5678–5684.
19 Connat, J.L., Diehl, P.A., Dumont, N., Carminati, S. and Thompson, J.J., Effects of exogenous ecdysteroids on the female tick *Ornithodoros moubata*: induction of supermolting and influence on oogenesis. Z. Angew. Entomol., 96 (1983) 520–530.
20 Matsuda, H., Kawaba, T., Yamamoto, Y. and Ogawa, S., Effect of ecdysterone on experimental artherosclerosis in rabbits. Folia Pharmacol. Jap., 70 (1974) 325–339.
21 Uchiyama, M. and Otaka, T., Phytoecdysones and protein metabolism in mammalia. In W.J. Burdette (ed.), Invertebrate Endocrinology and Hormonal Heterophylly, Springer-Verlag, Berlin, Heidelberg, New York, 1974, pp. 375–400.
22 Modde, J.F., Lafont, R. and Hoffmann, J.A. Ecdysone metabolism in *Locusta migratoria* larvae and adults. Int. J. Invert. Reprod. Dev., 7 (1984) 161–183.
23 Janocko, L. and Hochberg, R.B., Estradiol fatty acid esters occur naturally in human blood. Science, 222 (1982) 1334–1336.
24 Wigglesworth, K. and Rees, H.H., Poster presentation at the Congress on 'Biosynthesis, Metabolism, Mode of Action of Invertebrate Hormones', Strasbourg, 1983.
25 Dübendorfer, A., Oral presentation at the 6. Ecdysone Workshop, Szeged, 1983.
26 De Reggi, M.L., Hirn, M. and Delaage, M.A., Radioimmunoassay of ecdysone: an application to *Drosophila* larvae and pupae. Biochem. Biophys. Res. Commun., 66 (1975) 1307–1315.