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CASTOR-BEAN ACID LIPASE CATALYSED HYDROLYSIS OF TRIACYLGLYCEROLS DOES NOT INVOLVE C-2 → C-1,3 TRANSACYLATION

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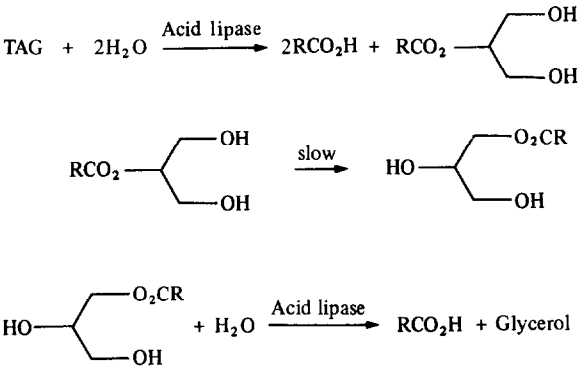
Key Word Index—*Ricinus communis*; Euphorbiaceae; castor-bean; acid lipase; lipolysis; triacylglycerol hydrolases.

Abstract—The hydrolysis of a series of triacylglycerol analogs catalysed by castor-bean acid lipase was studied at 30° and pH 4.20. *Iso*-propyl esters underwent lipolysis, thus refuting the mechanistic proposition that hydrolysis at C-2 in triacylglycerols occurs via a slow transfer of the acyl moiety from C-2 to either C-1 or C-3, followed by enzymic hydrolytic action.

The seeds of *Ricinus communis* are known to contain several lipases active at acidic pH values, which are collectively termed as 'castor-bean acid lipase' [1]. Ory *et al.* [2] studied the lipolytic activity of a partially purified acid lipase at pH 4.20 and reported that no reaction was observed for substrates such as *n*-hexyl oleate, 2-hexyl oleate or 2,3-butylidene dioleate. From their results, the authors proposed that the following mechanism should take place for full triacylglycerol (TAG) enzymic hydrolysis:



This communication describes the results of experiments from which the above mechanism was shown not to occur.

The enzyme preparation of Ory [3] was easily produced as a white powder containing 58 ± 4% protein. Polyacrylamide gel electrophoresis showed six bands and electrofocusing revealed a minimum of four isoenzymes of acid lipase. These observations are similar to those of Anan'eva *et al.* [4] who found 5 lipolytically active fractions on Sephadex G-100 gel chromatography. Details on the extract composition will be discussed elsewhere.

The action of acid lipase was studied on *n*-propyl, *iso*-propyl, 1,2-propylidene and 1,3-propylidene acetates and butyrates, as well as on triacetin and tributyrin in the form of emulsions stabilized in 5% aqueous gum arabic. The kinetics were measured at 30° and pH 4.20 by potentiometric titration of the liberated RCO_2H. All substrates used did undergo hydrolysis (Table 1).

We believe that the difference between the results of Ory and ours probably arises from the physical state of the substrates in the reaction mixtures; Ory used the reactants grossly suspended in an aqueous medium. It should be noted that lipolytic enzymes act on substrates which are constituents of hydrophobic interphases [5–7].

The system reactivity varies from *iso*-propyl esters through the TAG analogs up to the full TAG structures. Since the reactivity observed is not a linear combination of the reaction velocities of the *n*-propyl and *iso*-propyl substrates, the results suggest that the active site topology for these enzymes evolved to give an optimal fit for TAG substrates.

The fact that *iso*-propyl esters underwent hydrolysis, shows that the mechanism suggested by Ory and collaborators [2] for hydrolysis at C-2 in TAG is not an obligatory pathway, although a lower reactivity certainly exists at C-2, but due exclusively to steric effects.

EXPERIMENTAL

Plant material. R. communis seeds were collected at this University campus in January 1983.

Extraction and isolation. The method of ref. [3] was followed.

Table 1. Initial reaction velocities (μmol/min) for the hydrolysis of a series of triacetin and tributyrin analogs at 30° and pH 4.20 (1.0 mmol of emulsified substrate and 2.0 mg of castor-bean acid lipase in 4.0 ml total vol)

Alcohol	Acetate	Butyrate
n-Propanol	0.19 ± 0.04	0.37 ± 0.03
iso-Propanol	0.12 ± 0.03	0.17 ± 0.02
1,2-Propanodiol	0.14 ± 0.01 (di)	1.03 ± 0.04 (di)
1,3-Propanodiol	0.28 ± 0.02 (di)	1.20 ± 0.01 (di)
Glycerol	0.42 ± 0.01 (tri)	2.50 ± 0.07 (tri)

The only difference was the use of Et_2O rather than petrol for oil separation from the aq. seed extract.

Kinetic experiments were carried out at 30° and pH 4.20 as follows: 2.0 ml of the acid lipase soln (1.0 mg/ml, 10 mM Tris buffer, pH 8.1) was mixed with 2.0 ml of the substrate emulsion (0.50 mmol/ml in 5% aq. gum arabic). Enough 0.1 M HOAc was then added to adjust the pH to 4.20. The amount of free acid generated was followed by potentiometric titration with 50 mM NaOH as described in ref. [8]. Initial reaction rates were determined according to Boeker [9] as:

$$v_i = \lim_{P \to 0} \Delta P/\Delta t.$$

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FURTHER SILPHINENE DERIVATIVES FROM *CINERARIA GEIFOLIA* VAR. *GLABRA*

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Key Word Index—*Cineraria geifolia* var. *glabra*; Compositae; sesquiterpenes; silphinene derivatives.

Abstract—A reinvestigation of the sesquiterpenes from the aerial parts of *Cineraria geifolia* var. *glabra* afforded eight new silphinene derivatives. One of these compounds had been isolated previously but its structure had not been elucidated with certainty. The stereochemistry of a corresponding isovalerate from *Callilepis salicifolia* has to be revised.

INTRODUCTION

A keto diester had been isolated from *Cineraria geifolia var. glabra* (tribe Senecioneae) [1]. However, the decision between two possible structures was difficult and therefore only a preliminary structure was reported [1]. A reinvestigation of this compound and some minor related sesquiterpenes allowed a clear assignment of the structures. In addition to the acetoxy angelate **1**, which was isolated previously, three further diesters (**2–4**), the hydroxy ester **5** and the monoesters **6–8** were obtained.

RESULTS AND DISCUSSION

The ^{1}H NMR spectral data of **1** (Table 1) were close to those of a ketone obtained by oxidation of a hydroxy isovalerate from *Callilepis salicifolia* [2]. Careful spin decoupling allowed the assignment of all signals. The resulting sequences of the carbons C-1–C-3 and C-7, C-11, C-10–C-9 (C-15) showed that we were dealing with a derivative of silphinene [3]. The stereochemistry, however, could only be established by NOE difference spectroscopy (Table 2). Also the relative position of the ester group could be assigned from the NOEs. Thus irradiation of H-12 caused a clear effect with the methyl signals of the angelate residue, while H-13 and H-14 showed NOEs with the acetoxy methyl. Furthermore, inspection of a model indicated that due to the quasi-axial orientation of the 11β-acetoxy group the H-5 signal was shifted downfield. As H-11 showed a clear NOE with H-7 and H-10α the β-orientation of the acetoxy group was established. The assignment of H-10α followed from the observed small coupling with H-9. As H-15 showed an NOE with H-1 the α-orientation of H-9 was settled. Similarly all other orientations followed from the observed NOEs. The ^{13}C NMR signals (see Experimental) also agreed nicely with the proposed structure.

The ^{1}H NMR spectra of **2–4** (Table 1) were nearly identical with that of **1**, only the typical signal of the