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Studies on Catechol Esters

Part III. Hydrolysis of *o*-Hydroxyphenyl Acid Succinates; Competing Intramolecular Nucleophilic and General Base Catalysis[1]

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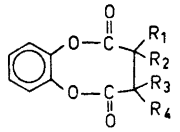
pH-Rate profiles for the hydrolysis of a series of methylsubstituted catechol monosuccinates and for catechol monophthalate have been determined at 25.0°C in water containing 11 % (by volume) acetonitrile. At values of pH below 8, the esters were found to hydrolyze *via* intramolecular carboxylate ion catalysis, whereas at values of pH above 8, a change in mechanism was recognized and the esters were found to hydrolyze *via* intramolecular general base catalysis from the *ortho* phenolate group. The effect from increased methyl group substitution in the succinic acid moiety (the *gem*-dimethyl effect) is reflected as dramatic increases in the rates of the intramolecular carboxylate ion catalyzed reaction, while the rates of the intramolecular general base catalyzed reaction are retarded by increased steric hindrance for the attack of a water molecule at the ester carbonyl carbon atom. The mechanism for the intramolecular carboxylate ion catalyzed reaction is discussed in terms of a possible ring-chain tautomeric equilibrium of the protonated form of the catechol monosuccinate.

In order to understand the mechanism of enzyme action, intramolecular catalysis in model systems has been extensively studied during the last decade. In this way, the effect of several functional groups (*i.e.* $-OH$, $-NR_2$, imidazole, $-COOH$, *etc.*) believed to exert catalytic activity in enzymatic reactions, has been investigated in systems comprising reactives such as ester and amide.[2] Thus, it has been demonstrated that carboxylate ion participates in the hydrolysis of monoesters of dicarboxylic acids having good leaving groups, while those esters having poor leaving groups hydrolyze *via* neighboring carboxyl group participation.[2,3] Moreover, increased substitution in the dicarboxylic acid moiety of the ester has been found to cause dramatic increases in the rates of hydrolysis of the intramolecularly catalyzed reactions (the *gem*-dimethyl effect).[1,4]

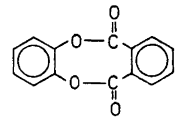
In our search for a blocking group against the enzymatic inactivation

of perorally administered catecholamines (*O*-sulphatation and *O*-methylation), we have undertaken an investigation to find out if the ester group, and particularly the succinoyl group, will fulfil the three requirements necessary for such a functioning: (1) the blocking group must be stable enough to acid to permit passage through the stomach, (2) it must be relatively easy to split off by means of enzymatic action in the blood plasma, and (3) if there are two blocking groups, both must be split off simultaneously or almost simultaneously in order to avoid undesired effects from or excretion of the monosubstituted derivative.

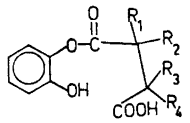
In an investigation on the kinetics of hydrolysis of a series of cyclic succinoylcatechols,[5] 3,4-dihydro-1,6-benzodioxocin-2,5-diones, *1a–d*, which



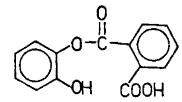
1a $R_{1-4}=H$ *c* $R_{1-4}=Me$
b $R_1=Me$, $R_{2-4}=H$



1d



2a $R_{1-4}=H$
b $R_1=Me$, $R_{2-4}=H$
c $R_{1,2}=Me$, $R_{3,4}=H$
d $R_{1,3}=Me$, $R_{2,4}=H$ (D,L)
e $R_{1,3}=Me$, $R_{2,4}=H$ (*meso*)
f $R_{1-3}=Me$, $R_4=H$
g $R_{1-4}=Me$



2h

have been synthesized [6] as model compounds for the more complicated catecholamine structures, we have verified that the rate of the first step in the hydrolysis of these esters is sensitive to steric hindrance in the acyl portion of the ester. Since it is known that the acylation and deacylation reactions of esterases by phenyl acetates are susceptible to steric hindrance in the acid moiety,[7] proper substitution in the succinic acid moiety in compounds like *1* should make it possible to regulate the rate of the enzymatic hydrolysis of the first step in a cyclic succinoylcatechol. Operation of the *gem*-dimethyl effect in the hydrolysis of the intermediate *o*-hydroxyphenyl acid succinate, *2*, should then increase the rate of the intramolecularly catalyzed hydrolysis of the second step. Consequently, the two ester groups will be split off almost simultaneously.

This report describes the kinetics of hydrolysis of a series of *o*-hydroxyphenyl acid succinates,[6] *2a–h*, in 11 % acetonitrile/water solution.

RESULTS AND DISCUSSION

The pH-rate profiles for the esters *2a*, *d*, *e*, *g*, and *h* are given in Fig. 1 and those for the esters *2b*, *c* and *f* in Fig. 2. Relevant rate constants are summarized in Table 1.

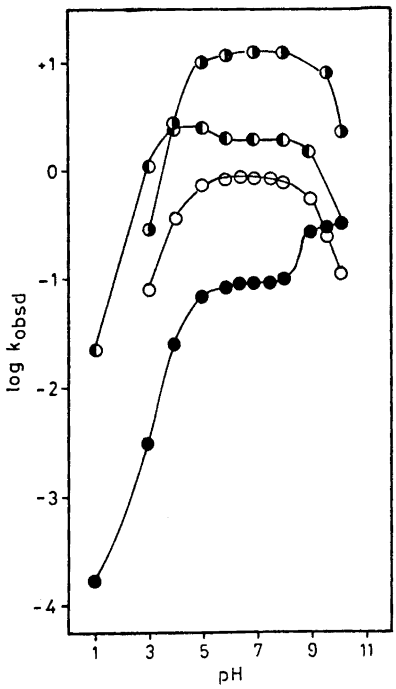


Fig. 1. pH-Rate profiles for the catechol monosuccinates *2a* ●, *2d* ○, *2g* ◑ and for catechol monophthalate *2h* ◐ at 25.0°C in water containing 11 % (by volume) acetonitrile.

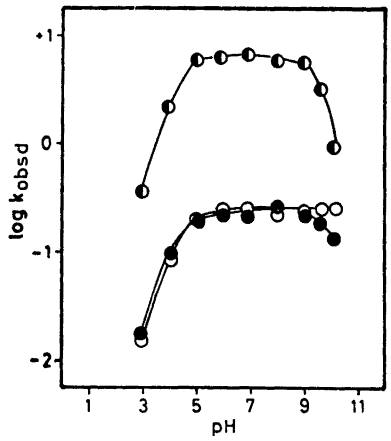


Fig. 2. pH-Rate profiles for the catechol monosuccinates *2b* ○, *2c* ● and *2f* ◐ at 25.0°C in water containing 11 % (by volume) acetonitrile.

A common feature of the pH-rate profiles for the esters *2a*–*h* is found between values of pH 2 and *ca.* 8, which part of the curve can be described by eqn. (1):

$$k_{obs} = \alpha\, k_{\alpha} = k_{\alpha} \frac{K_{app}}{(K_{app} + [H^+])} \qquad (1)$$

In eqn. (1) α represents the degree of ionization of the carboxyl group and k_{α} is the rate constant for hydrolysis *via* intramolecular carboxylate ion attack, *i.e.* at the plateau of the pH-rate profile.

The kinetic pK_{app}-values for the carboxyl groups in the esters *2a*–*h* were determined by plotting k_{obs} *vs.* $k_{obs}\,[H^+]$ (Eadie plot) and the values found were in the range 4.3 ± 0.2. This is in agreement with other investigations

Table 1. Values of k_α for the hydrolysis of substituted aryl hydrogen succinates (phthalate) and wavelengths of kinetic measurements.

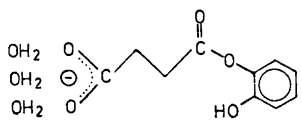
Ester	k_α min^{-1}	λ nm	k_{obs} min^{-1}, pH 10.1	λ nm
2a	0.093 (0.076)[a]	280.0	0.33	230.0
2b	0.24	280.0	0.25	230.0
2c	0.22	280.0	0.13	230.0
2d	0.84	280.0	0.11	230.0
2e	0.83	280.0	–	–
2f	6.8	280.0	0.93	230.0
2g	12.3	280.0	2.3	230.0
2h	1.9	250.0	0.31	247.0
2h	0.54[b]	300.0	–	–
Phthalic anhydride	0.54	300.0	–	–
Phenyl hydrogen succinate	0.085[a]	–	–	–
o-Methoxyphenyl hydrogen succinate	0.065 (0.072)[a]	280.0	0.067	(extrapol.)
9	–	–	0.32	230.0

[a] Ref. 17. [b] Rate of disappearance of the intermediate formed.

on similar systems and also with the value of p$K = 4.52$ obtained from titration data for monoesters of succinic acid.[10]

The influence of alkyl substituents in the acid moiety of monophenyl esters of glutaric acid has previously been investigated[4] and it has been found that introduction of one or more alkyl groups in the acyl portion of the ester strongly increases the rate of the intramolecularly catalyzed reaction. The *gem*-dimethyl effect has also been found to operate in the cyclization reactions of succinic acids,[11] and from Figs. 1 and 2 it is evident that also for the esters *2a – h*, increased degree of alkyl substitution increases the rate of the intramolecular carboxylate ion catalyzed reaction. Thus, it is found that the permethyl substituted ester *2g* hydrolyses *ca.* 130 times faster than *2a*.

Together with the steric compression of the ground state toward the transition state, caused by accomodation of alkyl substituents in the carbon chain (the *gem*-dimethyl effect), which will increase the rate of hydrolysis, solvation of the carboxyl anion by the solvent (water) is supposed to work in the opposite direction by allowing a larger population of transoid conformations of the ester (see *3*).



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This suggestion [12] is supported by the higher rates of phenyl phthalate esters, where the cisoid conformation of the carboxyl groups is locked, compared to phenyl succinates in aqueous solution, while in 1.0 M H_2O in DMSO the rate constants are essentially identical. The different behavior of the succinate esters in the two solvents can be rationalized in terms of a higher population of cisoid conformations of the succinates in the DMSO solvent, owing to lessened solvation of the carboxyl anion.

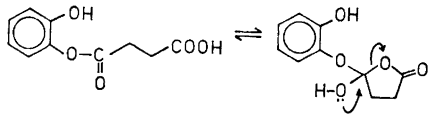
The higher rates of the esters *2f* and *g*, compared to their lower homologues (see Table 2) may also be explained in terms of lessened solvation of the

Table 2. Values of the logarithms of the relative rates $\left(\frac{k_\alpha}{k_{\alpha^\circ}}\right)$ for catechol monosuccinates.

Ester	log k_{rel}
2a	0.000
2b	0.412
2c	0.374
2d, e	0.951
2f	1.865
2g	2.122
2h	1.311

carboxylate anion, owing to a high degree of steric compression so that solvent molecules cannot be interposed. Thus, desolvation of the nucleophile may not be a portion of the energy barrier for the reaction.

Further support for the high degree of steric compression in the esters *2f–h* is found in the ability of those compounds to undergo ring-chain tautomerism (eqn. 2). Thus, in the case of esters *2f*, *g*, and *h*, after silylation with *N,O*-bis(trimethylsilyl)-acetamide, both the ring and chain forms as their silyl derivatives are present in the reaction mixture (MeCN) as demonstrated by GC/MS.[9]

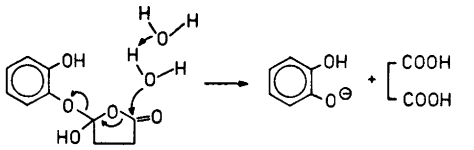


(2)

2

If ring-chain tautomerism is operating among the ester *2a–h*, hydrolysis of the ring form *4* may require general base catalysis (eqn. 3).

This mechanism involves a slow proton transfer step and hence the rate should be decreased in D_2O solvent. However, no solvent deuterium isotope effect has been found for any of the esters *2a–h*. If an uncatalyzed pathway



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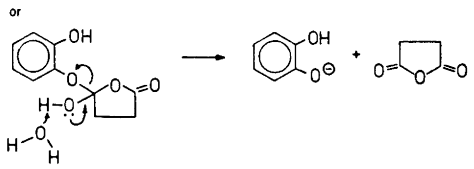


(reversed reaction in eqn. 2) is considered, which is reasonable owing to the presence of an adjacent electron-withdrawing carbonyl group, this should give back the chain form of the ester, *i.e.*, *2*, which *per se* is unreactive under the actual experimental conditions.

Thus, we believe that the intramolecular nucleophilic catalysis of hydrolysis of the esters *2a–h* takes place *via* the chain form *5b* of a possible ring-chain tautomeric equilibrium (see eqn. 4).

A further support for this mechanism is that in the hydrolysis of *2h* the existence of phthalic anhydride as an intermediate was unequivocally demonstrated (see Table 1).

Also the excellent log-log correlation between the rate constants for the intramolecularly catalyzed hydrolysis of the esters *2a–h* and the equilibrium constants for the corresponding succinic acids [11] (line B in Fig. 3), or the rate

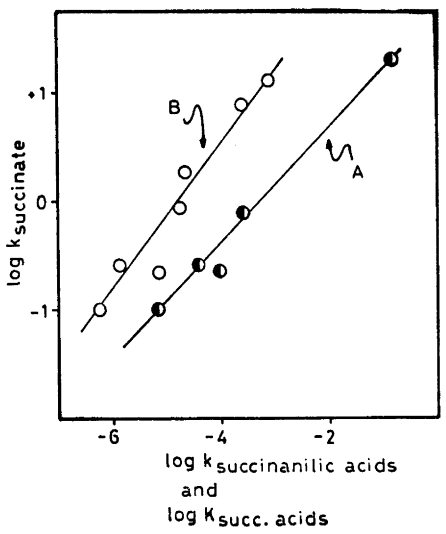
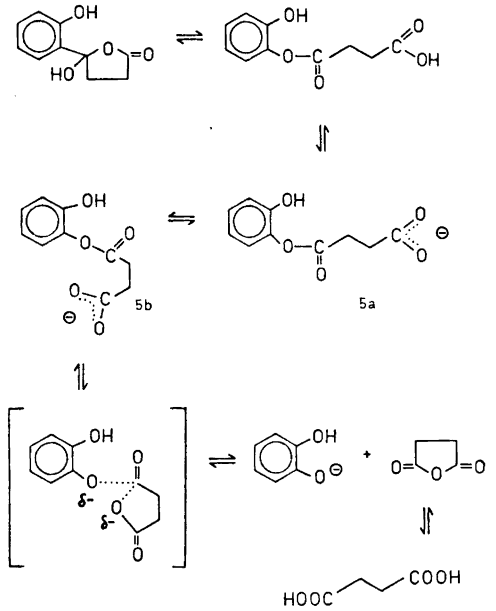


Fig. 3. Plot of the logarithm of the rate constants for the intramolecularly catalyzed hydrolysis of succinanilic acids (line B, values at 25.0°C), and of the logarithm of the equilibrium constants for a series of succinic acid (line A, values at 60°C) *vs.* log k_α for the catechol monosuccinates *2a–g*.

constants for the intramolecularly catalyzed hydrolysis of succinanilic acids [13] (line A in Fig. 3), (the rate constant for phthalamic acid was taken from Ref. 14), are consistent with a mechanism where formation of a dicarboxylic acid anhydride is the rate determining step.

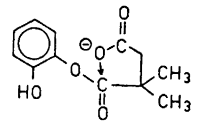
The unsymmetrically substituted esters *2b*, *c*, and *f* are supposed to have the most substituted carbon atom closest to the ester carbonyl group, owing to the nature of the method used for their preparation.[6]

This may explain the unexpectedly slow hydrolysis of the ester *2c*, for which attack on the ester carbonyl group by the carboxylate ion is hindered by the two *gem*-methyl groups on the adjacent carbon atom (see *6*). This is also reflected in the bad fitting to the lines A and B of the point for the ester



(4)

2c (Fig. 3) where comparison is made with compounds which undergo attack on a carbonyl group having no methyl substituents on the adjacent carbon atom (see *7* and *8*).



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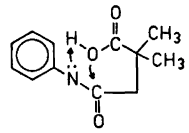

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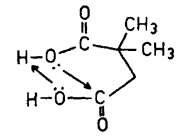

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The similarity in rate constants for the DL and *meso* forms of the 2,3-dimethyl substituted ester, *i.e. 2d* and *e*, must be interpreted with care because epimerization may well have taken place during the synthesis of the *meso* form.[15]

The "hump" in the pH-rate profile for *2h* (*ca.* pH 4, Fig. 1) results from difficulties in obtaining stable isosbestic points during the kinetic measurements, owing to simultaneous formation and hydrolysis of phthalic acid monophosphate.[16]

For ester *2a*, E_a was determined (Table 3) and it was found to have activation parameters comparable to those obtained for other phenyl acid glutarates and succinates.[17]

Table 3. Values of k_α and activation parameters [a] for the hydrolysis of catechol monosuccinate *2a*, pH 6.90.

Ester	k_α min^{-1}	t°C	$\Delta H^\ddagger$ kcal/mol	$T\Delta S^\ddagger$ at 25°C
2a	0.093	25.0	16.9	−5.3
	0.23	35.0		
	0.89	48.0		

[a] See Frost, A. A. and Pearson, R. G. *Kinetics and Mechanism*, Wiley, New York 1953, pp. 95–97.

All the esters *2a–h* were found to be very sensitive to traces of basic impurities in the MeCN used as solvent for the stock solutions. Acetonitrile usually contains basic contaminants in the concentration range $10^{-5}-10^{-6}$ M[18] and it was found that *2a* decomposed with a rate $k_{obs}=0.5$ min^{-1} in untreated MeCN. However, after passing the MeCN through acid form alumina, the esters were found to be stable for several weeks in this solution. Addition of 2×10^{-5} M piperidine to a base free MeCN solution of the ester *2a* caused decomposition of the ester with a rate $k_{obs}=5\times10^{-2}$ min^{-1}.

Intramolecular general base catalysis. The hydrolytic behavior of the esters *2a–h* at pH values above 8 (Figs. 1 and 2) may be interpreted in terms of intramolecular general base catalysis of the attack of a water molecule at the ester carbonyl carbon atom from the *ortho* phenolate group. Thus, it is found that for *2a*, the pH-rate profile shows an increase in rate at pH values above 8 and a second plateau at pH > 9 which coincides with the plateau found in the pH-rate profile of catechol monoacetate [1] (Fig. 4).

Since the kinetic pK_{app} values for *2a* and *9* are identical (8.6) within the limits of experimental error, this finding strongly supports the assumption that these compounds hydrolyze according to the same mechanism at pH > 8. Catechol monobenzoate (*10*) and *9* have previously been shown to undergo hydrolysis with intramolecular general base catalysis from the adjacent *o*-phenolate group.[19]

Furthermore, the solvent deuterium isotope effect for *2a* and *10* are identical ($k_{H_2O}/k_{D_2O}=1.8$ [20]) which points to a similarity in mechanism.

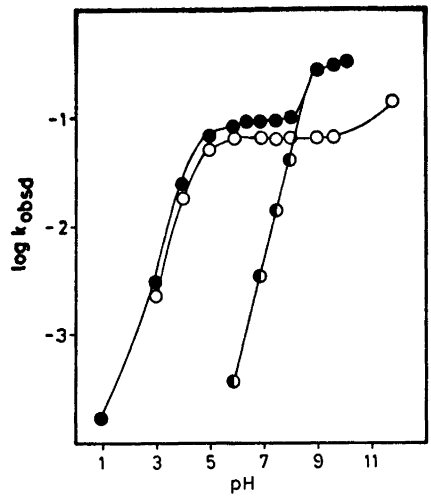
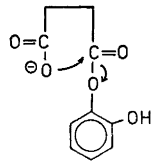


Fig. 4. pH-Rate profiles for catechol monosuccinate *2a* ●, *o*-methoxyphenyl hydrogen succinate ○, and catechol monoacetate *9* ◐, at 25.0°C in water containing 11 % (by volume) acetonitrile.

The absence of general acid catalysis in the hydrolysis of the catechol monosuccinates is evident from the small variation in the hydrolysis rates of the monosuccinates of phenol, *o*-methoxyphenol, and catechol (*2a*) (Table 1). As can be seen from the pH-rate profiles of esters *2b*–*g* (Figs. 1 and 2), methyl substitution in the succinic acid moiety decrease the rate of the intramolecular general base catalyzed reaction. The retardation of this mechanism is due to increased steric hindrance for the general base catalyzed attack of a water molecule at the ester carbonyl carbon atom. Moreover, the pH-rate profile for *2a* shows that the intramolecular carboxylate catalysis must be unimportant when intramolecular general base catalysis by the adjacent *o*-phenolate group is operating. This is not unexpected in view of the electrostatic repulsion introduced in the dianion of *2a*. It is, however, possible that the degree of intramolecular carboxylate ion participation at pH > 8 increases as the succinic acid moiety becomes more substituted, which of course will make the participation of the adjacent *o*-phenolate group less efficient.

Thus, we have presented strong evidence for *competing* intramolecular nucleophilic and general base catalysed mechanisms in the hydrolysis of the catechol monosuccinates *2a*–*g* and the catechol monophthalate *2h*. These mechanisms can be represented by formulas *11* and *12*.



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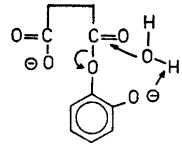

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Competing intramolecular nucleophilic and general (base) catalysis has also been found to take place in many other similar systems, where in many

cases the bell-shaped pH-rate profiles obtained had been interpreted as resulting from bifunctional catalysis. In all cases, however, it has been found [21] that only one group participated directly in the hydrolytic reaction and the decrease in rate observed at high pH values was shown to result from electrostatic and electronic inhibition resulting from the ionization of the non-participating functional group.

EXPERIMENTAL

Materials. Acetonitrile (Eastman analytical grade) was passed through alumina (Merck, acid form, activity grade I) to remove traces of basic impurities before use (*vide supra*). The deuterium hydroxide used (99.8 % isotopic purity) was obtained from CIBA. Buffer solutions (0.1 M, containing 10^{-5} M EDTA) were prepared from analytical grade chemicals and doubly distilled water.

Kinetic measurements. The kinetics of hydrolysis of compounds *2a–h* were studied in 11 % MeCN/H_2O utilizing citrate-phosphate buffers (pH 3 – 5), potassium phosphate buffers (pH 5 – 8), borate-phosphate buffers (pH 8 – 9), and borate buffers (pH 9 – 10). No attempt was made to control ionic strength in the experiments since the rates were not significantly affected by the addition of 1 M NaCl. No change in pH was found during the kinetic runs and buffer catalysis was not observed. Triplicate runs were made at each pH and the rate constants were usually reproducible within ± 5 % of the mean value. The rates were measured spectrophotometrically with a Unicam SP 800 C spectrophotometer, equipped with an external Texas Servowriter II, by following the increase in absorption of the released catechol. The esters were first dissolved in MeCN and then 0.2 ml of this stock solution was added from a micro pipet to the preheated buffer solution (2.0 ml) in the thermostated cuvette (25 ± 0.1°C). The solution was then stirred by blowing argon through the solution for a few seconds. In the case of esters *2f* and *g*, the preheated buffer solution was rapidly injected from a syringe into the thermostated cuvette, which contained 0.2 ml of the ester stock solution. At least five runs were made at each pH value. The rates were generally followed to at least 75 % of completion and pseudo-first-order rate constants (k_{obs}) were obtained by the method of Guggenheim.[8] Constant temperature was maintained by circulating water at 25 ± 0.1°C from a HETO Ultrathermostat (Birkerød, Denmark). At the end of each reaction, the UV-spectrum of the products was identical to that of an authentic mixture of catechol and the expected acid. It was usually not possible to measure the rate of hydrolysis at values of pH higher than 10, owing to autoxidation of the liberated catechol.

Activation parameters were calculated from plots of log *k vs.* ($1/T$) from data obtained from kinetic runs carried out at three different temperatures (25.0, 35.0, and 48.0°C). Owing to the instability of esters *2f* and *g*, no pure crystalline products could be obtained, but both the kinetic behavior and mass spectrometric analysis of the TMS-derivatives [9] of the crude esters agreed with the proposed structure.

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