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THE REACTION OF 3-OXO FATTY ACID ESTERS WITH TREHALOSE DERIVATIVES IN AN ALKALINE MEDIUM.

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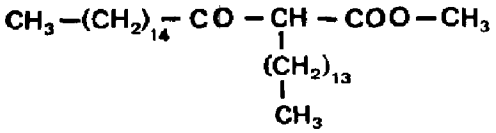
Summary : The primary 6'-hydroxyl function of the 6-O-[3-oxo-acyl]-α,α-trehalose participates in its base-catalyzed retro-Claisen decomposition.

In a preceding paper[1], we have described attempts of transesterification of the methyl 3-oxo fatty acid ester I with either α,α-trehalose or aliphatic α-glycols, catalyzed by potassium carbonate[2]. Cleavage esters according to a retro-Claisen reaction were obtained, whereas the normal products of transesterification were formed when either methyl α-D-glucopyranoside or monoalcohols or (1,x)aliphatic glycols (x > 2) were used as acceptors. The induction of the retro-Claisen reaction by aliphatic α-glycols was explained by an intramolecular interaction between the remaining free hydroxyl group and the 3-oxo function in a mono-3-oxo acyl ester of the corresponding glycol formed in an intermediary step. This paper presents a study of the interaction of I with either α,α-trehalose or modified trehaloses in order to define some particularities of the intramolecular retro-Claisen reaction.

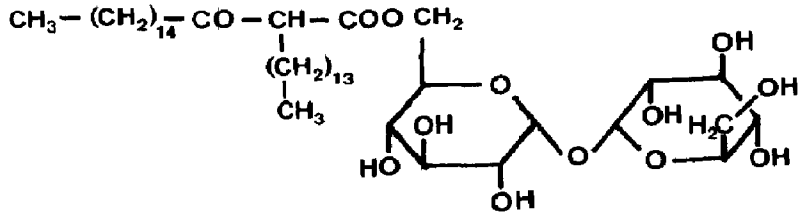
The intermediary formation of α,α-trehalose 3-oxo-esters was observed when I and α,α-trehalose were left to react in an aprotic medium (DMF) containing K_2CO_3 as a catalyst, at 80° under vacuum (80 torr) during a short time. Using a catalyst concentration of 30 mM and a molar ratio 1/4 between I and α,α-trehalose, a 27 % yield of

trehalose 3-oxo esters and a 14 % yield of trehalose palmitates were obtained after 30 minutes reaction time. After 1 h 30, the respective yields were 15 % and 56 % and after 5 h, not any 3-oxo ester of trehalose could be detected, whereas the yield of palmitate esters reached to 95 %. Decreasing the potassium carbonate concentration slowed down the retro-Claisen reaction more quickly than the esterification one, so that a higher intermediary yield of trehalose 3-oxo esters could be obtained (up to 40 % after 1 h 30 reaction time with a catalyst concentration of 15 mM) but palmitate esters were always formed (20 %).

A change in the basicity of the catalyst affected more strongly the reaction pattern. The addition of dicyclohexyl-18 crown-5 to potassium carbonate dramatically changed the process since palmitone, resulting from a decarboalkoxylation, was the only product which could be detected. A similar reaction was observed when cesium carbonate replaced potassium carbonate. In the opposite, the use of the less basic sodium carbonate catalyst decreased the speed of both the retro-Claisen and decarboxylation reactions so greatly that only traces of palmitate esters and palmitone could be detected even after very long reaction times (4 days), whereas the 3-oxo acyl esters of α,α-trehalose were obtained in a relatively good yield (up to 40 %). In despite of the long reaction time, this catalyst was used for the synthesis of 3-oxo acyl esters of α,α-trehalose from ^{14}C-labelled I since the unreacted methyl ester I was quantitatively recovered in the absence of side reaction products, and could be utilized again for successive runs. Under these last conditions, the main transesterification product of I with α,α-trehalose is the 6-O- [3-oxo-acyl] isomer II as demonstrated by the examination of the mass spectra of the TMS derivatives[3] and by comparison with an authentic sample of the 6-derivative[4]. In the K_2CO_3 alkaline conditions [10], the compound II gave 6-O-palmitoyl trehalose as the main cleavage product together with the 6,6'-dipalmitate ester and other unidentified poly-acylated trehaloses.



I



II

To determine which specific hydroxyl group of II would induce, by an intramolecular interaction, the retro-Claisen reaction leading to palmitate esters, we successively reacted several modified trehalose derivatives with I in the presence of K_2CO_3 as a catalyst. The results are presented in table I. The main conclusions were the following :

i) The suppression of the free 6'-primary hydroxyl group in α,α-trehalose inhibited the retro-Claisen reaction and the main reaction products were the 6-O- [3-oxo-acyl] esters of the modified trehaloses[9].

ii) The presence of a bulky protective group on the 6'-position strongly reduced the yield of the transesterification product, which is an indication of the existence of conformations leading the 6 and 6' functions in a close proximity.

iii) The modification of the stereochemistry at the glycosidic linkage also modifies the possibilities of interaction, and the β,β-trehalose behave as methyl glucopyranoside, since mostly transesterification products are formed.

ivi) When α,α-trehalose was used, the 6-monopalmitoyl ester was the main reaction product although the 6,6'-bis-palmitoyl ester would be the expected one resulting from an intramolecular retro-Claisen cleavage. This result could be explained by successive transesterification reactions of the latter compound, leading to an equilibrium mixture in which the 6-O-palmitoyl ester was the main product. Decreasing the sugar/ I ratio led to the formation of a complex mixture containing mainly mono, di and poly palmitoyl trehalose esters.

The 6-O- [3-oxo acyl] -α,α-trehalose II is a constituent of Corynebacterium diphtheriae cells[4] which is biosynthesized from palmitic acid through a carboxylation step[14], and catabolized into palmitic acid by an another way (unpublished results). The chemical cleavage of II in a slightly alkaline medium led us to assume that the enzymatic cleavage also occurs by an intramolecular reaction and that the role of trehalose in the biosynthetic pathway could be to locate, at a favourable distance for condensations, a palmitoyl and a tetradecylmalonyl groups esterifying the 6 and 6'-positions of a hypothetic trehalose diester.

Table 1 : Products formed during reaction of I and modified trehaloses[10].

Modified trehaloses	unreacted I (%)	3-oxo-esters (products of transesterification) (%)	mono-palmitoyl esters (retro-Claisen products) (%)	other esters (%) (11)	palmitone (%)
6-O-trityl-α,α-trehalose[7]	83	14	0	3	0
6-O-methyl-α,α-trehalose[8]	64	22	4	10	-
6-deoxy-trehalose[6]	67	26	2	5	-
α,α-trehalose[12]	0	0	95	traces	5
β,β-trehalose[13]	15	37	2	30	16

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7. This product was synthesized by a partial tritylation of α,α-trehalose. The monotrityl derivative was isolated by chromatography of the peracetyl derivatives, followed by alkaline methanolysis of the acetyl groups.
   $[\alpha]_D^{20}$ = 115°5 (c=0.3, methanol)- F.D. mass spectrum $(M + Na)^+$ at m/e 607
8. This product was obtained by a diazomethane methylation catalyzed by BF_3[5] of the 2,3,4, 2',3',4',6'-heptadecyl-α,α-trehalose, followed by alkaline methanolysis of the acetyl groups.
   $[\alpha]_D^{20}$ = + 122° (c=0.4, methanol) - F.D. mass spectrum $(M + Na)^+$ at m/e 379
   The absence of isomerisation reactions was controlled by the identification of glucose and 6-O-methyl glucose as hydrolysis products.
9. The separation of the different isomers was realized by TLC of the pertrimethylsilyl derivatives. The identification of the structure of the 6-acylated derivative was done by mass spectrometry[3].
10. Experimental procedure : a mixture containing the methyl 3-oxo ester I (0.4mM), the selected modified trehalose (0.4mM) and 20 mg of anhydrous and finely grounded potassium carbinate in 5 ml of anhydrous dimethyl formamide was stirred at 80° during 6 hours under a vacuum of c.a. 80 torr. After a chloroform extraction, the different products were separated by a silicic acid column chomatography.
11. The bis- or poly-acylated sugar molecules were estimated as "other esters".
12. The molar ratio sugar/I in this experiment was equal to 4.
13. The "other esters" are a mixture of poly-3-oxo-acylated -β,β-trehalose derivatives.
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