' Le BOP ' Reagent and Imidazole for Selective *O*-Acylation of Trehalose

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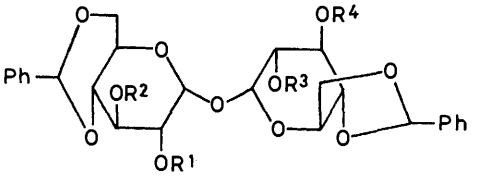
Benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (' Le BOP ' reagent) in combination with imidazole provides a very mild system for selective esterification of polyhydroxy-compounds. An example is given showing an improved selectivity towards position 2 of trehalose, leading to new derivatives of ' Cord-Factor.'

THE biological significance of fatty esters of trehalose has been widely demonstrated.[1] One of the most important, ' Cord-Factor ' (6-*O*-mycoloyl-α-D-glucopyranosyl-6'-*O*-mycoloyl-α-D-glucopyranoside) which is extracted from *Mycobacterium tuberculosis* cultures, exhibits cytotoxic properties, and inhibits Ehrlich's ascites growth. Chemical synthesis of this product has been achieved in the last few years,[2] and recently we synthesised an analogue.[3]

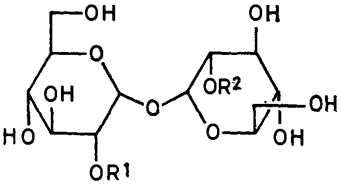
We decided to prepare unsymmetrical esters of trehalose on secondary positions in order to compare their biological properties with those of ' Cord-Factor ' and its analogues. Monosubstituted derivatives of trehalose, and especially trehalosamine [4] showed interesting properties. Moreover a paper issued during the course of our own work pointed out the importance of mono-esters at the 6-position of trehalose and sucrose [5] which possessed an antitumour effect against certain mice carcinomas.

We chose to protect primary hydroxy-groups by formation of an acetal ring between O-6 and O-4. Direct benzylidenation [6] of trehalose hydrate with benzaldehyde and zinc chloride readily afforded the symmetrical derivative (2). It was necessary to find a mild and selective method to esterify only one of the four free hydroxy-groups. A recent review [7] showed that the use of *N*-acylimidazoles seemed to furnish some improvements. Hence, the difference of reactivity between O-2 and O-3 should permit a selectivity in favour of the former. More recently, when we achieved this work, Gelas and Horton demonstrated that acetylation with *N*-acetylimidazole took place preferentially at O-6 and then at O-2.[8]

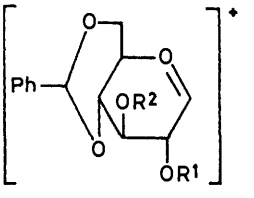


	R^1	R^2	R^3	R^4
(2)	H	H	H	H
(3)	Palm	H	H	H
(4)	Palm	Ac	Ac	Ac
(5)	Palm	H	Palm	H
(6)	Palm	Ac	Palm	Ac
(7)	Palm	Palm	Palm	H
(8)	Palm	Palm	Palm	Ac

Palm = $-\overset{\overset{O}{\|}}{C}-[CH_2]_{14}Me$



	R^1	R^2
(9)	Palm	H
(10)	Palm	Palm



	R^1	R^2
(11)	H	H
(12)	Palm	H
(13)	Palm	Palm

Most of the reported methods used acyl chlorides and imidazole to prepare *N*-acylimidazoles. This method was not reliable for *e.g.* functionalized or fatty acids. In relation to our experience of peptide synthesis we used the active-ester method to prepare acylimidazole. We have shown that carboxylic acids and protected amino-acids reacted with Le BOP reagent [9] (1) to yield an active ester which reacted with phenol to afford

phenolic ester.[10] In the case of other alcohols it is necessary to add a catalyst. Imidazole or hydroxybenzotriazole itself were effective, as demonstrated by Klausner *et al.* for the synthesis of depsipeptides.[11]

RESULTS

In related work, we were able to prepare benzyl esters of Boc-amino-acids in almost quantitative yields, using Le BOP and imidazole; similarly, preliminary tests in the D-glucose series had demonstrated that imidazole catalysis was the more effective.[12] The Scheme summarizes the principle of our method for the generation of *N*-acylimidazoles.

The reaction was performed at room temperature in three different solvents with different stoicheiometric ratios of acid : alcohol. After the time indicated in Table 1, the solvent was removed under reduced pressure and the crude residue chromatographed on a silica gel column, which allowed the separation of two main products accompanied by a small amount of a third one (Table 1).

TABLE 1

Formation of trehalose 2-palmitate (3), 2,2′-dipalmitate (5), and 2,2′,3-tripalmitate (7)

Solvent	Acid : alcohol ratio	Time/h	Products * (% yield) (3)	(5)	(7)
Acetone	1 : 1	48	31	0	0
Acetone	2 : 1	48	74	20	5
Acetonitrile	1 : 1	48	34	27	0
Acetonitrile	2 : 1	48	44	20	0
DMF	1 : 1	24	49	21	0
DMF	2 : 1	24	32	24	6
HMPA	1 : 1	100	43	17	0

* Yields calculated on the basis of sugar used.

Structural Assignments.—The degree of substitution was determined by high-resolution mass spectrometry, the molecular weight being confirmed by elemental analysis. The sites of esterification were determined by 250-MHz ^{1}H n.m.r. spectroscopy of the corresponding acetylated derivative obtained by conventional acetylation (acetic anhydride–pyridine). This acetylation promoted separation of the signals of the H-2, H-2′, H-3, and H-3′ protons from the signals of the other cyclic protons.

The results obtained by mass spectroscopy are summarized in Table 2.

The first most-polar component was the tripalmitoyl derivative (7) as shown by its molecular ion at *m/e* 1 232; the presence of both oxonium ions (13) and (12) confirms this fact.

The medium-polarity product (5) exhibited the molecular ion at *m/e* 994 and a fragment-ion at *m/e* 489. The absence of the oxonium ion (13) showed the symmetry of this dipalmitate. The less-polar ester was the monopalmitate (3) as indicated by the molecular ion at *m/e* 740 and the oxonium ions (11) and (12).

It was now important to determine the position of

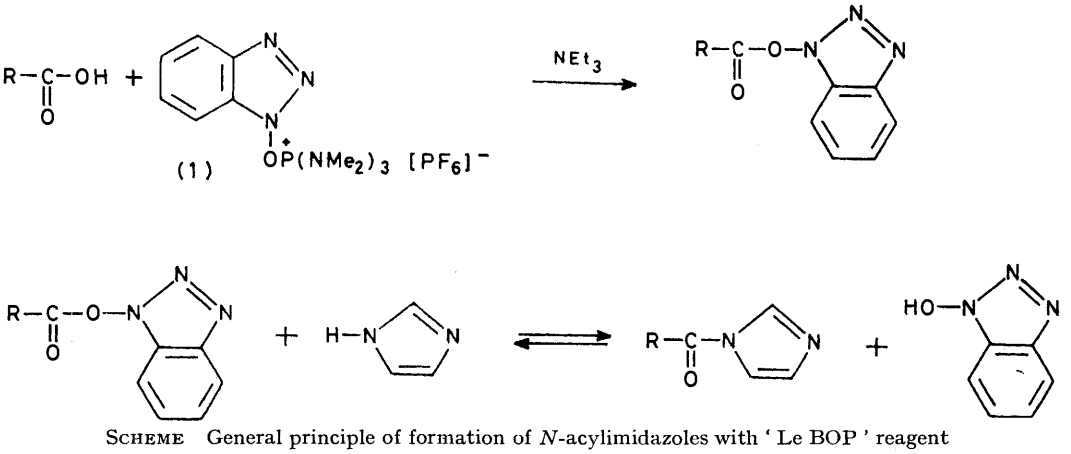
TABLE 2

Significant peaks in the mass spectra of peracetylated palmitates (*m/e* values)

Ester	*M*+	Oxoniums ions (11)	(12)	(13)
(3)	740	251	489	
(5)	994		489	
(7)	1 232		489	727

esterification of these three derivatives. Examination of the n.m.r. spectra of the acetylated derivatives (4), (6), and (8) was performed and the signals of the H-3, H-3′, H-2, and H-2′ protons were accurately analysed. At 250 MHz, the chemical shifts of H-3 and H-3′ are slightly different if O-3 and O-3′ are esterified by acetate and palmitate. The same difference was observable for H-2 and H-2′ under the same conditions.

It is clear from the Figure that the product (6) is completely symmetric whereas (4) and (8) are dissymmetric. Moreover in the case of compound (4), the single signal for H-3 and H-3′ suggested that the both O-3 and O-3′ were acetylated, and the splitting of H-2 and H-2′ (both double doublets) indicated that (4) was esterified only at O-2 by a palmitate residue. Thus the structure of (8) was determined to be a 2,2′,3-tri-*O*-palmitoyl derivative because of the difference of chemical shifts between H-3, H-3′ and H-2, H-2′, attributable to the presence of a palmitoyl residue at position 3. Esterification at positions 2, 3, and 3′ was excluded for chemical resaons, the reactivity of O-2, being greater than that of O-3′.



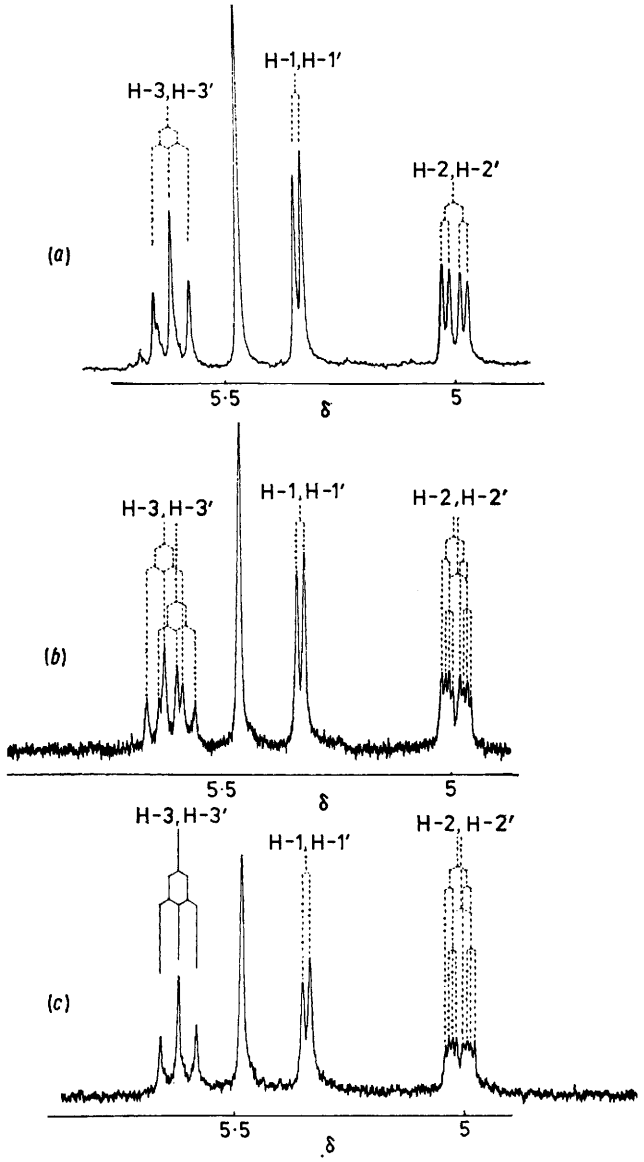
SCHEME General principle of formation of *N*-acylimidazoles with ' Le BOP ' reagent

DISCUSSION

Examination of Table 1 shows that the change of solvent does not alter considerably the course of the reaction. It proceeds smoothly in every case and

specially in HMPA. DMF seems to be a solvent of choice for this reaction as previously demonstrated by Klausner *et al.*[11]

This method is naturally much more useful than those using the reaction of acyl chlorides on imidazole,[13] since it starts directly from the free carboxylic acid. It is even more useful than that using carbonyldi-imidazole [14] as a greatly improved selectivity was obtained. We attribute this selectivity to the following feature: if Le



250-MHz ^{1}H N.m.r. spectra of the ring protons of peracetylated palmitates: (*a*) 2,2'-dipalmitate (6); (*b*) 2,2',3-tripalmitate (8); (*c*) 2-palmitate (4)

BOP was used without any catalyst, no reaction was observed except the formation of the active palmitic ester of hydroxybenzotriazole. In the presence of imidazole this active ester produced a little quantity of highly active acylimidazole, in equilibrium with the heterocyclic ester: the gentle liberation of acylimidazole was responsible for the improved selectivity.

Removal of benzylidene protecting groups has been performed by mild acid hydrolysis. Refluxing in ethanol–water containing hydrochloric acid readily afforded the fully deprotected palmitates (9) and (10). The biological properties of these compounds are now under investigation.

EXPERIMENTAL

General Methods.—^{1}H N.m.r. spectra were routinely obtained at 60 MHz using a Perkin-Elmer R 12 B spectrometer. High-field n.m.r. spectra of acetylated derivatives were obtained using a Cameca 250 operating at 250 MHz in the continuous-wave mode. All the spectra were recorded in $CDCl_3$, with chemical shifts (δ) downfield from $SiMe_4$ as internal standard. High-resolution mass spectra were obtained on a AEI SM9 spectrometer. T.l.c. was conducted on pre-coated Merck plates and developed by spraying with 50% H_2SO_4 and heating at 150 °C. Preparative column chromatography were conducted with Kieselgel 60 (Merck) eluting with ether–light petroleum mixtures. Melting points were measured on a Kofler block. Optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter. Solvent evaporation was carried out under reduced pressure at temperature below 40 °C.

Typical Procedure for Ester Preparation.—4,6;4',6'-di-*O*-benzylidene-α,α'-trehalose (dried by azeotropic distillation with pyridine) was dissolved in the solvent with 1 equiv. of palmitic acid and 1.1 equiv. of Le BOP reagent. After complete dissolution on stirring, 1.1 equiv. of imidazole followed by 1.1 equiv. of NEt_3 were added. The mixture was stirred during the time indicated in Table 1. The solvent was then evaporated and the crude residue dissolved in chloroform was chromatographed on a column of silica gel. Slow elution allowed separation of (7), (5), and (3) in that order. N.m.r. spectra at 60 MHz are not described, but all showed the presence of benzylidene and of palmitoyl residues and were consistent with the structure.

4,6;4',6'-*Di*-O-*benzylidene*-2,2'-3-*tri*-O-*palmitoyl*-α,α'-*trehalose* (7) had m.p. 113 °C (from ether–hexane); $[\alpha]_D^{20}$ +53.9° (*c* 0.1, $CHCl_3$); R_F 0.73 [ether–hexane (55 : 45)]; *m/e* 1 232 (M^+), 727 (oxonium), 489 (oxonium), and 239 (palmitoyl residue) (Found C, 72.11; H, 10.01. Calc. for $C_{74}H_{120}O_{14}$: C, 72.07; H, 9.74%).

4,6;4',6'-*Di*-O-*benzylidene*-2,2'-*di*-O-*palmitoyl*-α,α'-*trehalose* (5) had m.p. 194 °C; $[\alpha]_D^{20}$ +80.7° (*c* 0.3, $CHCl_3$); R_F 0.57 [ether–hexane (55 : 45)]; *m/e* 994 (M^+), 489 (oxonium), and 239 (palmitoyl) (Found C, 70.10; H, 9.11. Calc. for $C_{58}H_{90}O_{13}$: C, 69.97; H, 9.13%).

4,6;4',6'-*Di*-O-*benzylidene*-2-O-*palmitoyl*-α,α'-*trehalose* (3) had m.p. 138 °C; $[\alpha]_D^{20}$ +72.6° (*c* 0.2, $CHCl_3$); R_F 0.15 [ether–hexane (55 : 45)]; *m/e* 740 (M^+ − 16), 489 (oxonium), 251 (oxonium), and 239 (palmitoyl) (Found C, 66.51; H, 8.09. Calc. for $C_{42}H_{60}O_{12}$: C, 66.63; H, 8.01%).

Acetates (4), (6), and (8) were prepared from pure derivatives by acetylation with acetic anhydride–pyridine followed by conventional work-up. Purifications were achieved by short-column chromatography using ether–light petroleum (40 : 60). N.m.r. spectra are shown in the Figure.

Benzylidene Removal.—This was achieved as follows: 1 mM of the blocked derivative was suspended in 10 ml of ethanol containing 1 ml of water. Two drops of 12N hydrochloric acid were added and the mixture was refluxed until all material had dissolved (20—30 min). T.l.c.

monitoring [AcOEt–MeOH (9 : 1)] showed complete removal of the benzylidene group. Solvent was thoroughly evaporated. Four additions and evaporations of water (5 ml) followed by addition and evaporation of ethanol and toluene afforded a syrup which readily crystallized from acetone. In this way the following two compounds were prepared.

2-O-*Palmitoyl*-α,α′-*trehalose* (9) had m.p. 166 °C (acetone); $[\alpha]_D^{20}$ +131.1° (*c* 0.23, EtOH); R_F 0.1—0.3 [AcOEt–MeOH (9 : 1)]; $\nu_{max.}$ 3 500 (OH) and 1 750 cm^{-1} (ester) (Found: C, 55.78; H, 8.98. Calc. for $C_{28}H_{52}O_{12}$: C, 57.82; H, 8.94%).

2,2′-*Di*-O-*palmitoyl*-α,α′-*trehalose* (10) had m.p. 192 °C (acetone); $[\alpha]_D^{20}$ +102.9° (*c* 0.2, EtOH); R_F 0.5—0.6 [AcOEt–MeOH (9 : 1)]; $\nu_{max.}$ 3 500 (OH) and 1 750 cm^{-1} (ester) (Found: C, 64.62; H, 10.01. Calc. for $C_{44}H_{82}O_{13}$: C, 64.54; H, 10.02%).

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