Improved Synthesis of 'Cord Factor' Analogues

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Treatment of trehalose or sucrose with triphenylphosphine, di-isopropyl azodicarboxylate, and palmitic acid results in the formation of the corresponding 6,6'-dipalmitates–analogues of 'cord factor'–in good yield under exceptionally mild conditions.

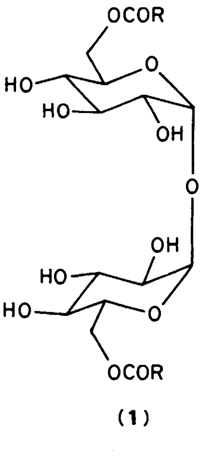
Certain strains of bacteria form long filaments or serpentine-like 'cords'. Light petroleum (b.p. 40—60 °C) extraction of the bacteria leads to the isolation of a lipid fraction called 'cord factor'. The extracted bacteria are still viable but no longer form cords.[1] Cord factor is a glycolipid: the 6,6'-dimycolic acid ester of α,α-D-trehalose (**1**, R = $C_{83}H_{163}O$). Mycolic acids (after the bacteria–mycobacteria) are long-chain (*ca.* C_{84}), α-branched, β-hydroxy acids.[1,2]

More recently, interest in cord factor has been intensely renewed because of its *immunostimulant properties* and its *anti-tumour activity*.[1—3] Interestingly, various analogues of cord factor such as trehalose 6,6'-dipalmitate (**1**, R = $C_{15}H_{31}$) and sucrose 6,6'-dipalmitate (**2**, R = $C_{15}H_{31}$) also exhibit a number of the biological effects of cord factor, but generally larger doses of the analogues are necessary.[1]

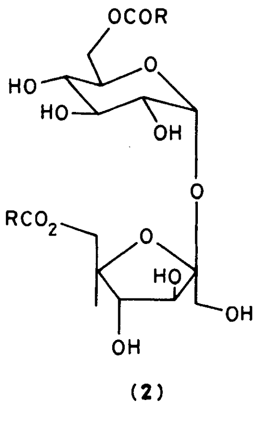
Although cord factor and a number of analogues have been synthesised in recent years, the methods used have either been cumbersome (involving the synthesis of selectively protected carbohydrate precursors followed by standard acylation with a fatty acid chloride) or have given abysmal yields.[1]

We now report a simple, mild, and selective procedure for the synthesis of cord factor analogues [(**1**, R = $C_{15}H_{31}$) and (**2**, R = $C_{15}H_{31}$)] in good yield, directly from the free disaccharides. The following procedure is illustrative: trehalose (1 g), triphenylphosphine (2.07 g), and palmitic acid (1.95 g) were dissolved in dry dimethylformamide (DMF) (14 ml) and cooled to 0 °C. Di-isopropyl azodicarboxylate (DIAD, 1.65 g) was added dropwise to the cooled, stirred solution over a period of 15 min. The mixture was stirred at room temp. for 24 h and then evaporated to dryness under vacuum (bath temperature <50 °C). The residue was taken up in chloroform, filtered, and chromatographed on silica gel (Kieselgel 60, 70–230 mesh; $CHCl_3 : MeOH : MeCO_2H : H_2O$ 79 : 11 : 8 : 2) to give the crude product (1.4 g, 59%). Recrystallisation from acetone gave the dipalmitate [(**1**, R = $C_{15}H_{31}$), 1.31 g, 55%], m.p. 158.5—160 °C, lit.[4] 154—158 °C; $[\alpha]_D$ +78 ° (*c* 1, $CHCl_3$), lit.[4] +80 ° (*c* 1, $CHCl_3$).

A similar procedure with sucrose gave an identical crude yield (59%) of sucrose 6,6'-dipalmitate. Recrystallisation from ethanol gave pure crystalline dipalmitate [(**2**, R = $C_{15}H_{31}$), 0.86 g, 36%], m.p. 109 °C lit.[5] 107—109 °C; $[\alpha]_D$ +40 ° (*c* 1, $CHCl_3$), lit.[5] + 40 ° (*c* 0.5, pyridine).



(**1**)



(**2**)

The mechanism of the Mitsunobu reaction[6] has recently been shown[7,8] to involve the formation of dialkoxytriphenylphosphorane intermediates. The selectivity observed in these reactions is presumably a result of the preferential formation of such phosphoranes on the primary hydroxy groups. Moreover, nucleophilic displacement of triphenylphosphine oxide by the carboxylate anion would be expected to take place preferentially at the primary carbon atoms. In the case of sucrose, the 1'-position being neopentyl-like, is considerably more hindered than the 6- and 6'-positions. Interestingly we have observed that the 6-position is considerably more reactive than the 6'-position of sucrose in these reactions. This is in constrast to the silylation of sucrose with t-butylchlorodiphenylsilane where it is the 6'-position that is the most reactive.[9]

Thus, treatment of sucrose under the conditions described above with palmitic acid (1.2 equiv.), triphenylphosphine (1.4 equiv.), and DIAD (1.4 equiv.) in DMF for 76 h at room temp. gave 6-palmitoylsucrose (6-*O*-hexadecanoyl-α-D-glucopyranosyl-β-D-fructofuranoside, 0.8 g, 47%) as a very hygroscopic, waxy solid (softening point 60 °C, melting finally at 210 °C), lit.[5] 100—104 °C; $[\alpha]_D$ +40 ° (*c* 1, pyridine), lit.[5] 43.4 (*c* 0.25, pyridine). The product was shown to be the 6-palmitate rather than the 6'-palmitate by hydrolysis with invertase to give fructose (but not glucose). The 6'-palmitate would not be hydrolysed by invertase.[10] This assignment was also supported by ^{13}C and ^{1}H n.m.r. data.

In conclusion, a simple mild procedure for the synthesis of cord factor analogues is presented. The method should be generally applicable to a range of synthetic immunostimulants which could prove of considerable value in the treatment of immunodepressed patients. The method may also have important uses in the antigenic determinant area, for example in the selective modification of cell-surface oligosaccharides.

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