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**Note**

# Diamide pseudo cord-factors: bis-*N*-acyl derivatives of 6,6′-diamino-6,6′-dideoxy-α,α-trehalose

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The unusual biological properties of cord factor (6,6′-di-*O*-mycoloyl-α,α-trehalose) have been known for many years (for recent reviews on cord factor, see refs. 1 and 2). For examining the structure–biological-function relationships of cord factor, 6-*O*-mycoloyl derivatives of sucrose, methyl D-glucosides, -mannosides, -allosides, -galactosides, and D-glucitol were prepared by Asselineau and Kato[3]. The results showed[3] "the importance of the configuration at the asymmetric centers and the necessity of a definite conformation of the polar moiety of the molecule". Simpler 6,6′-diesters of α,α-trehalose have also been extensively studied[4–6].

This laboratory has recently been involved in the synthesis of various cord factors and pseudo cord-factors for a similar purpose: to examine the influence on biological activities of structural variations, not in the carbohydrate core, but in the groups linking the carbohydrate and the lipid substituents. The first paper in this series[7] described the synthesis of several pseudo cord-factors from (α-D-glucopyranosyluronic acid) (α-D-glucopyranosiduronic acid) ("trehalose-dicarboxylic acid"). Two types of pseudo cord-factor were described: the diester type (that we term "mirror" Ψ cord-factors) obtained from the dicarboxylic acid and 4-[4-(hexadecyloxy)phenyl] butanol, and the diamide type (termed "mirror amide" Ψ cord-factors) obtained from the dicarboxylic acid and several amines. In subsequent papers[8,9], natural cord-factors and analogs were synthesized from 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6,6′-di-*O*-(methylsulfonyl)-α,α-trehalose and lipid carboxylic acids.

We now describe the synthesis of "amide" pseudo cord-factors[7] from a suitably protected 6,6′-diamino-6,6′-dideoxy-α,α-trehalose derivative and lipid carboxylic acids.

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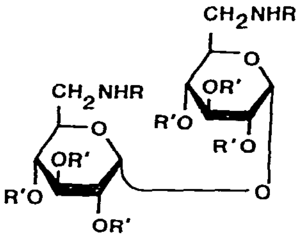
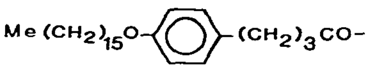
## DISCUSSION

Treatment of 6,6′-diamino-2,3,4,2′,3′,4′-hexa-*O*-benzyl-6,6′-dideoxy-α,α-trehalose[10] (**1**) with 4-[4-(hexadecyloxy)phenyl]butanoic acid in dichloromethane in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) under the conditions described in the literature[11] proceeded satisfactorily, and diamide **2** was obtained in good yield.



| | R | R′ |
|---|---|---|
| 1 | H | $CH_2Ph$ |
| 2 | a | $CH_2Ph$ |
| 3 | b | $CH_2Ph$ |
| 4 | Mycoloyl | $CH_2Ph$ |
| 5 | a | H |
| 6 | b | H |
| 7 | Mycoloyl | H |

a =


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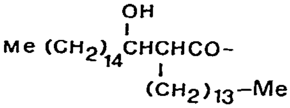

$$\mathrm{Me(CH_2)_{14}\overset{\overset{\displaystyle OH}{|}}{C}H\underset{\underset{\displaystyle (CH_2)_{13}{-}Me}{|}}{C}HCO{-}}$$

Residual carboxylic acid was removed by treatment with an ion-exchange resin, and further purification of the product was achieved by column chromatography. Although acylation of hydroxyl groups in the presence of DCC is unlikely, the utilization of the hexabenzyl ether **1** was especially useful, owing to its high solubility in dichloromethane. Similarly, treatment of the diamine **1** with corynomycolic acid[12] and DCC gave the corresponding diamide **3** in good yield; it was isolated as a homogeneous syrup after ion-exchange treatment and column chromatography. Another benzylated diamide pseudo cord-factor (compound **4**) was obtained when the hexa-*O*-benzyl diamine **1** was acylated with mycolic acid. The product was purified as just described.

Difficulties were encountered in the catalytic debenzylation of compounds **2**, **3**, and **4** owing to the low solubility of these compounds, as well as of the corresponding debenzylated products, in ethanol. Utilization of 1:1 ethyl acetate–ethyl alcohol, as recently described in a paper on the synthesis of cord factor and analogs[9], was successful only for diamide **2**, for which debenzylation was complete, and the pure, crystalline product **5** was isolated in satisfactory yield. For the diamide derivatives **3** and **4**, the best results were obtained when hydrogenolysis was conducted in 3:2 dichloromethane–ethyl alcohol that also contained a small proportion of hydrochloric acid. The products (**6** and **7**) were purified by column chromatography, and isolated as

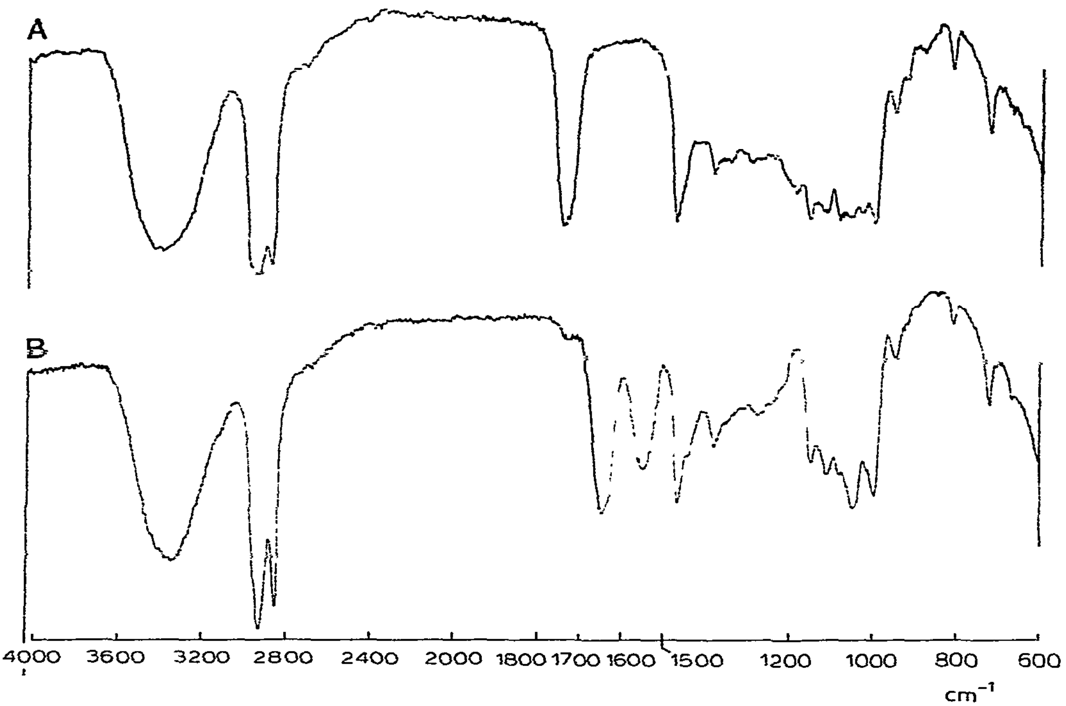

Fig. 1. Infrared spectra of (A) a true cord factor (6,6′-dicorynomycoloyl-$\alpha,\alpha$-trehalose) and (B) the analogous "amide" pseudo cord-factor (6). Absorption bands characteristic of the trehalose core at 809 cm$^{-1}$ and in the region 1000–1150 cm$^{-1}$ are evident in both spectra. Instead of ester absorption at 1735 cm$^{-1}$ characteristic of the true cord factors, the amide pseudo-cord factor has absorptions at 1550 and 1650 cm$^{-1}$, *in accord with its structure.*

homogeneous waxes in satisfactory yield. The chromatographic behavior of the final products **5**, **6**, and **7** resembled that of the corresponding diester derivatives (cord factors and analogs) already described[9]. The microanalytical data for compounds **2**–**6** were in accord with the structures assigned. For compound **7** the C and H values were somewhat high, probably because the *M. bovis* "mycolic acid" used in the synthesis is heterogeneous[13], and the exact empirical formula of this compound is not yet known.

The foregoing three, purified amide pseudo cord-factors (above) were homogeneous by t.l.c. and gave i.r. spectra in accord with their amide structures. They were not examined by $^1$H-n.m.r. spectrometry: n.m.r. spectra of the highly lipidic trehalose derivatives are rarely informative, because in $CDCl_3$ the carbohydrate protons are unrelaxed and yield broad, clumped peaks[7]. Fig. 1 compares the i.r. spectrum of 6,6′-di-*O*-corynomycoloyltrehalose (a true cord–factor) with that of the corresponding amide $\Psi$ cord-factor **3**. It is evident that, excepting the carbonyl absorptions, the spectra are nearly identical: the band at 809 cm$^{-1}$ and the series of closely spaced peaks between 1000 and 1150 cm$^{-1}$ are characteristic of the trehalose moiety of cord factor as described before[14]. In the spectrum of **3** (Fig. 1b), the ester absorption at about 1735 $cm^{-1}$ (Fig. 1a), characteristic of the true cord factor, is missing; instead amide I and II absorptions are seen at about 1550 and 1650 $cm^{-1}$.

"Amide" pseudo cord-factors[7,15] based upon 6,6′-diamino-6,6′-dideoxytrehalose have not been prepared before. Goren and Jiang[16] were able to obtain meager yields of presumed compound **5** by direct acylation of "diamino-α,α-trehalose" with the corresponding acid chloride in the presence of aqueous sodium hydrogencarbonate[17]. The diamine was prepared by the sequence α,α-trehalose → 6,6′-di-*O*-tosyl-α,α-trehalose → 6,6′-diazido-6,6′-dideoxy-α,α-trehalose → 6,6′-diamino-6,6′-dideoxy-α,α-trehalose. However, it is well known that direct tosylation of this carbohydrate affords a complex mixture of tosylated derivatives[18]. The scheme described herein avoids the structural uncertainties inherent in the earlier approach, and allows taking advantage of the carbodiimide-promoted amidation reaction. Indeed, direct acylation of the "diamino-α,α-trehalose" with acid chlorides precludes utilization of the more interesting β-hydroxylated mycolic acids, which are representative of natural cord-factors. Moreover, direct acylation afforded a complex mixture of products that were separated only with difficulty.

The function of natural cord-factors in preparations having antitumor activity[4,15] is largely unknown (see, however, McLaughlin *et al.*[19]). Trehalose mycolates and related glycolipids have putatively been implicated as carriers for the transfer of newly synthesized mycolic acids to the growing mycobacterial cell-wall[14,20]. If the antitumor activities of α,α-trehalose mycolates depend on a similar behavior (namely, a transacylation, perhaps mediated by microsomal enzymes, in which mycoloyl groups are transferred to tumor-cell surfaces and thus render then antigenically distinct from the "normal" tumor-cell surface), it is less likely that a similar transacylation could be accommodated by an *amidated* mycolic acid.

## EXPERIMENTAL

For general methods, see ref. 10.

*2,3,4,2′,3′,4′-Hexa-O-benzyl-6,6′-dideoxy-6,6′-di-{4-[4-(hexadecyloxy)phenyl]-butanoylamino}-α,α-trehalose* (**2**). — To a solution of the diamine **1** (190 mg) in dichloromethane (5 mL) were added 4-[4-(hexadecyloxy)phenyl]butanoic acid (265 mg) and *N,N′*-dicyclohexylcarbodiimide (DCC, 150 mg). The mixture was stirred for 2 h at room temperature, and acetic acid (0.2 mL) was then added. Evaporation of the mixture gave a solid residue that was treated with AG-MP 1 ($OH^-$) ion-exchange resin (Bio-Rad Laboratories) suspended in 1:1 chloroform–methanol for a few minutes. The resin was filtered off and washed with 1:1 chloroform–methanol, and the filtrate was evaporated to give a white solid. The residue was extracted with ether, and the insoluble material (probably 1,3-dicyclohexylurea) was filtered off, and washed with ether. Evaporation of the combined filtrate and washings gave the crude product, which was purified by column chromatography. Elution with 2:1 hexane–ethyl acetate removed some minor byproducts. Elution with 3:2 ethyl acetate–hexane, followed by 2:1 ethyl acetate–hexane, gave pure **2**; yield, 197 mg (55%); $[\alpha]_D^{24}$ +40.7° (*c* 1, chloroform).

*Anal.* Calc. for $C_{106}H_{144}N_2O_{13}$: C, 76.96; H, 8.77; N, 1.69. Found: C, 77.15; H, 9.04; N, 2.05.

*2,3,4,2',3',4'-Hexa-O-benzyl-6,6'-di-(corynomycoloylamino)-6,6'-dideoxy-α,α-trehalose* (**3**). — A solution of the diamino compound **1** (78 mg) in dichloromethane (3 mL) was treated with corynomycolic acid[12] (150 mg) in the presence of DCC (95 mg) as described for compound **2**. The crude product was treated with AG-MP-1 ($OH^-$) resin as already described. The 1,3-dicyclohexylurea was removed by addition of ether and filtration, and the filtrate was evaporated to a syrup that was applied to a column of silica gel. The pure product was eluted with 3:2 hexane–ethyl acetate and isolated as a syrup in 76% yield (124 mg); $[\alpha]_D^{24}$ +28.6° (*c* 1.1, chloroform).

*Anal.* Calc. for $C_{118}H_{184}N_2O_{13}$: C, 77.07; H, 10.08; N, 1.52. Found: C, 76.99; H, 10.00; N, 1.56.

*2,3,4,2',3',4'-Hexa-O-benzyl-6,6'-dideoxy-6,6'-di-(mycoloylamino)-α,α-trehalose* (**4**). — The diamino derivative **1** (98 mg) was treated with mycolic acid (300 mg) (*M. bovis*, AN5) (kindly provided by the late Dr. R. Toubiana) in dichloromethane (5 mL) in the presence of DCC as already described. The crude product was purified by chromatography on silica gel. Elution with 3:1 hexane–ethyl acetate yielded a minor byproduct. Continued elution with the same solvent system, followed by 2:1 hexane–ethyl acetate, afforded **4** (231 mg, 63%). An analytical sample was obtained by rechromatography of a portion on silica gel, with 1:1 ether–hexane as the eluant; $[\alpha]_D^{24}$ +24.3° (*c* 1.2, chloroform).

*Anal.* Calc. for $C_{222}H_{384}N_2O_{13}$: C, 81.05; H, 11.76; N, 0.85. Found: C, 80.94; H, 11.77; N, 0.94.

*6,6'-Dideoxy-6,6'-di-{4-[4-(hexadecyloxy)phenyl]butanoylamino}-α,α-trehalose* (**5**). — The benzylated diamide **2** (128 mg) was dissolved in 1:1 dichloromethane–ethyl alcohol (60 mL), and hydrogenolyzed in the presence of 10% palladium-on-carbon catalyst (120 mg) at 50 $lb.in.^{-2}$ for 5 h. The catalyst was filtered off, and washed with chloroform, and the filtrate and washings were combined and evaporated to give a solid residue. Ethyl alcohol was added, and the crystalline material was filtered off; yield 47 mg (54%). It was recrystallized from ethyl alcohol to give an analytically pure sample; m.p. 140–145°, $[\alpha]_D^{24}$ +43° (*c* 1, chloroform).

*Anal.* Calc. for $C_{64}H_{108}N_2O_{13}$: C, 69.03; H, 9.77; N, 2.51. Found: C, 68.88; H, 9.80; N, 2.49.

*6,6'-Di-(corynomycoloylamino)-6,6'-dideoxy-α,α-trehalose* (**6**). — The 6,6'-diamido derivative **3** (105 mg) was dissolved in a mixture of dichloromethane (30 mL) and ethyl alcohol (20 mL). Hydrochloric acid (2M, 2 mL) and 10% palladium-on-carbon catalyst (110 mg) were added, and the mixture was hydrogenolyzed at 50 lb. $in.^{-2}$ for 5 h. The catalyst was filtered off and washed with chloroform, and the combined filtrate and washings were evaporated, giving a residue that was chromatographed on silica gel. Elution with 25:1 chloroform–methanol, followed by 15:1 chloroform–methanol, removed minor by-products. Elution with chloroform–

methanol (7:1 and 5:1) afforded pure **6**; yield 46 mg (62%); $[\alpha]_D^{24}$ +41.6° (*c* 1, chloroform).

*Anal.* Calc. for $C_{76}H_{148}N_2O_{13}$: C, 70.32; H, 11.49; N, 2.15. Found: C, 70.21; H, 11.68; N, 2.18.

*6,6'-Dideoxy-6,6'-di-(mycoloylamino)-α,α-trehalose* (**7**). — The benzylated diamide (**4**) (66 mg) was dissolved in dichloromethane (30 mL) and ethyl alcohol (20 mL), and hydrogenolyzed in the presence of palladium-on-carbon catalyst and hydrochloric acid as described for compound **6**. The product, which was almost pure, was purified by column chromatography. Elution with 20:1 chloroform–methanol removed a minor byproduct. Continued elution with the same solvent system, followed by chloroform–methanol (9:1 and 7:1), yielded pure **7**; yield, 31 mg (56%); $[\alpha]_D^{24}$ +26.4° (*c* 1, chloroform).

*Anal.* Calc. for $C_{180}H_{348}N_2O_{13}$: C, 78.65; H, 12.76; N, 1.02. Found: C, 78.07; H, 12.16; N, 1.14.

## ACKNOWLEDGMENTS

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