Carbohydrate Research, 209 (1990) 181–189
Elsevier Science Publishers B.V., Amsterdam

Synthesis of alkyl [(alkyl 6-deoxy-α-D-*gluco*-heptopyranosyl-uronate) 6-deoxy-α-D-*gluco*-heptopyranosid]uronates, a novel type of mirror pseudo cord factor

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(Received June 20th, 1990; accepted for publication July 27th, 1990)

ABSTRACT

The 1-octyl, 1-pentadecyl, 1-hexadecyl, 1-heptadecyl, and 1-octadecyl diesters of (6-deoxy-α-D-*gluco*-heptopyranosyluronic acid) 6-deoxy-α-D-*gluco*-heptopyranosiduronic acid, a new homolog of trehalosuronic acid, were prepared by two procedures. One procedure involved conversion of the peracetylated acid into its dichloride, reaction of the latter with the alkanols, and acid-catalyzed deacetylation of the products, whereas the other consisted of reaction of alkyl mesylates with the potassium salt of the unprotected acid.

INTRODUCTION

Cord factor, the toxic component of the cell walls of *Mycobacterium tuberculosis* and other mycobacteria, is α,α-trehalose 6,6′-dimycolate (**1**, R = various closely related, α-branched, β-hydroxylated lipid residues of formula weights near 2400)[1]. Analogous trehalose esters, containing the structurally similar nocardic and corynomycolic acids instead of mycolic acids, are found in some other microorganisms[2]. Trehalose and its 2-sulfate esterified with simpler fatty acids have also been isolated from mycobacteria and shown to possess interesting biological properties[3–5]; examples are the 6,6′-di-(hexadecanoate)[3] and a 2,3-di-*O*-acyl-2′-sulfate containing mainly hexadecanoyl and octadecanoyl groups[5]. Cord factor has been implicated in the mechanisms of bacterial pathogenicity and virulence (although definitive evidence is lacking)[1]. It causes granuloma formation[6], inhibits leucocyte migration[7], depresses NAD-dependent microsomal enzymes[8] and glycogen synthesis[9], and possesses immunostimulant and antitumor properties[10]. These diverse manifestations of biological activity call for the chemical synthesis of modified molecules potentially useful as biochemical probes for unraveling structure–activity relationships. One effort in this direction has been the synthesis of cord factor analogs in which the ester functionalities are regioinverted, as shown in formula **2**; such a dialkyl (hexosyluronate hexosiduronate) structure has been termed "mirror" pseudo cord factor[11]. We decided to synthesize compounds of type **3**, which represents dialkyl (6-deoxyheptosyluronate 6-deoxyheptosiduronates); from a structural point of view, **3** would mirror the natural cord factor (**1**) more closely than does **2**.

Although the ultimate objective of this project is to obtain **3** which bears mycolyl

0008-6215/91/$ 03.50

1
2
3
R = long-chain alkyl
and similar groups
4 R = H
5 R = Ac
6
7 R = Me
8 R = n-C8H17
9 R = n-C15H31
10 R = n-C16H33
11 R = n-C17H35
12 R = n-C18H37
13 R = n-C8H17
14 R = n-C15H31
15 R = n-C16H33
16 R = n-C17H35
17 R = n-C18H37

ester groups, we first set out to prepare simple glycolipids of this type, including the diester regioinversely isometric to natural trehalose 6,6′-di(hexadecanoate), as reported herein.

RESULTS AND DISCUSSION

The synthesis of (6-deoxy-α-D-*gluco*-heptopyranosyluronic acid) 6-deoxy-α-D-*gluco*-heptopyranosiduronic acid (**4**) and its hexaacetate **5** starting from α,α-trehalose has recently been described[12]. Treatment of **5** with oxalyl chloride in 1,2-dichloroethane furnished the diacid dichloride **6**. Although **6** was not purified and characterized by physical data, methanolysis of the crude material gave the crystalline dimethyl ester **7**, previously prepared[12] in two independent ways, thus verifying the success of the chlorination. Reaction of crude **6** with 1-octanol, 1-pentadecanol, 1-hexadecanol, 1-heptadecanol, and 1-octadecanol afforded the corresponding dialkyl esters **8**–**12** as analytically and spectroscopically pure oils in 81–96% yields based on **5**. (Only **12** crystallized in part, m.p. 37.5–39°). The next task was *O*-deacetylation of the products, to be accomplished, if possible, without significant uronic ester cleavage. For the dimethyl ester **7**, this has been achieved[12] by methoxide-catalyzed methanolysis (Zemplén), but that procedure was unsuitable for the higher esters. Deprotection of **8**–**12** succeeded by *p*-toluenesulfonic acid-catalyzed transesterification in 1,2-dichloroethane or benzene solution at 60°, using as acetyl acceptor an excess of the alcohol that constituted the uronic ester. However, the process was rather slow and somewhat ineffectual, requiring at least 4 days and, sometimes, recycling of incompletely deacetylated material and repeated recrystallizations of the chromatographically isolated products, which tended to diminish yields. Nevertheless, the fully deacetylated diesters were obtained crystalline and analytically pure in yields of 54 (**13**) and 28–36% (**14**–**17**).

In order to improve access to **13**–**17**, we considered other strategies that had proved efficient in cord factor synthesis, including the use of selectively removable trimethylsilyl[13] or benzyl[14] protecting groups for the sugar. Thus, an approach to the 2,3,4,2′,3′,4′-hexabenzyl ether of **4** was undertaken[12]; it led to the corresponding, per-*O*-benzylated dicarboxamide which, however, resisted conversion into the dicarboxylic acid. At this point, we were stimulated by the work of Liav and Goren[14], in which (benzyl-protected) 6,6′-dimesylates of α,α-trehalose and stereoisomers were condensed with potassium carboxylates, including mycolates, to furnish cord factor analogs. We found that the *unprotected* acid **4**, in the form of its dipotassium salt, is readily esterified by treatment with alkyl mesylates at 75° in dimethyl sulfoxide solution. This procedure afforded crystalline diesters in acceptable yields after chromatographic purification, namely 72% for **13** and 56–68% for **14**–**17**.

The structures of **8**–**17** were confirmed by elemental analysis and spectroscopic data. Although molecular-ion peaks were observed for **9**, **11**, **13**, and **14** only, the +f.a.b. mass spectrum of every compound showed a characteristic fragment, at m/z $0.5[M - 16]^+$, representing the glycosyl ion that results from rupture of the disaccharidic bond; in addition, daughter fragments originating from loss of 1–2 water

molecules were given by **13–17**. The ^{13}C-n.m.r. spectra of **8–12** in chloroform-*d* exhibited completely identical resonances for the sugar moiety and for several of the alkyl chain carbon atoms (Table I). Thus, the ester α-methylene group resonated at 65.1 p.p.m., and signals at 14.2, 22.7, and 32.0 p.p.m. were attributable to the terminal methyl and its two adjacent methylene groups; signals at 28.6 and 25.9 p.p.m. presumably belonged to the β- and γ-methylene groups as such carbons are slightly shielded when, in an alkane, a terminal methyl is replaced by a carbonyloxy substituent (in contrast to the strongly deshielded α-carbon)[15]. Methylene carbon atoms further inside the chains resonated at 29.45 ± 0.3 p.p.m., and it was in this narrow range that additional signals, both separated and coincident, appeared on going from **8** to the higher homologs. In particular, a signal at 29.73 p.p.m. characteristically grew in intensity in the sequence **9**→**10**→**11**→**12**. The picture was uniformly the same for the free-hydroxyl derivatives **13–17** in methanol-d_4 solution, with slightly and consistently higher shift values for all of the sugar and some of the alkyl carbon atoms (Table I). The ^{1}H-n.m.r. data (Table II) were completely identical for **8–12** as far as the carbohydrate proton signals were concerned, and the same, symmetrical, 2-proton multiplet for O-CH_2 (δ 4.0) and 3-proton triplet for the terminal methyl group (δ0.86) were seen. The spectra differed solely in the intensity ratios of these to a peak at δ1.23 for the bulk of the interior ester-chain protons. (Two of the latter resonated at δ1.55). Again, the pattern for **13–17** was entirely analogous (Table II).

Serological studies to determine possible cross reaction of **13–17** with *M. tuberculosis* antibodies are being undertaken in collaboration with Dr. A. Laszlo, Laboratory Centre for Disease Control, Health and Welfare Canada, Ottawa. Preliminary indications are that **14–17** show significant affinity for antibodies in sera from tuberculosis patients. Compounds **8** and **15–17** (**14** was not tested) were found to be capable of inducing the production of interleukin-6, and tumor necrosis factor in human monocytes. (Private communication by A. J. Ulmer, Division of Cellular Immunology, Forschungsinstitut Borstel, Borstel, F.R.G.)

EXPERIMENTAL

General methods. — Unless otherwise stated, the following solvent combinations (v/v) were used for column and thin-layer chromatography on silica gel: (*A*) 1:3 EtOAc–hexane; (*B*) 2:1, (*C*) 1:1, (*D*) 1:2, and (*E*) 1:3 ether–hexane; (F) 1:19, (*G*) 2:98, and (*H*) 1:99 MeOH–EtOAc; and (I) 4:5:30 H_2O–MeOH–EtOAc. Optical rotations were determined at ~25° with a Perkin–Elmer 241 polarimeter, with solutions in chloroform (*c* 0.6–1.0). I.r. data (ν_{max}) were recorded from thin films; only bands of particular constitutional significance are listed. Mass-spectral data (*m/z*) were obtained by the positive f.a.b. mode (using a glycerol matrix), unless otherwise indicated. The ^{1}H- and ^{13}C-n.m.r. data refer to spectra taken at 300 and 75.43 MHz, respectively, on a Varian XL-300 instrument; all ^{13}C peak assignements were supported by ADEPT experiments. The 1-alkanols were purchased from Aldrich Chemical Co.

*Alkyl [(alkyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-*D*-gluco-heptopyranosyluronate)*

TABLE I

^{13}C-N.m.r. chemical shifts (p.p.m.) at 75.43 MHz for **8–12** in chloroform-*d* and **13–17** in methanol-d_4 solution

Compound	*Sugar moiety*	*Alkyl chain* OCH_2	*Internal* CH_2 *groups*[a]								*Terminal* $CH_3CH_2CH_3$ *group*		
8	[b]	65.1	25.9,	28.6,	29.18,	29.21					31.8,	22.7,	14.15
9	[b]	65.1	25.9,	28.6,	29.3,	29.4,	29.55,	29.62,	29.69,	29.73	32.0,	22.7,	14.2
10	[b]	65.1	25.9,	28.6,	29.3,	29.4,	29.55,	29.62,	29.69,	29.73	32.0,	22.7,	14.2
11	[b]	65.1	25.9,	28.6,	29.3,	29.4,	29.55,	29.63,	29.70,	29.74	32.0,	22.7,	14.2
12	[b]	65.1	25.9,	28.6,	29.3,	29.4,	29.55,	29.62,	29.69,	29.73	31.9,	22.7,	14.15
13	[c]	65.7	26.8,	29.6,	30.00,	30.04					32.7,	23.4,	14.1
14	[c]	65.7	26.8,	29.6,	30.1,	30.15,	30.35,	30.5			32.8,	23.4,	14.1
15	[c]	65.7	26.8,	29.6,	30.1,	30.2,	30.4,	30.5			32.8,	23.45,	14.15
16	[c]	65.7	26.8,	29.6,	30.1,	30.45[d],	30.5				32.8,	23.4,	14.1
17	[c]	65.7	26.75,	29.55,	29.95,	30.1,	30.45				32.75,	23.4,	14.05

[a] The first two signals listed are tentatively assigned to C-2 and C-3. The values listed last and second to last for **9–12** represent two or more coincident signals, with the former increasing in relative intensity with growing chain length. [b] 90.9 (C-1), 71.8, 70.4, 69.0, and 66.3 (C-2,3,4,5), 36.6 (C-6), 169.8-169.6 (C-7 and 3 *Me* CO), 20.8-20.7 (3 *CO*Me). [c] 94.3 ±0.1 (C-1), 75.0, 74.6, 73.3, and 69.8 (C-2,3,4,5), 38.1 (C-6), 173.2 ±0.1 (C-7). [d] Shoulder at the strong signal at 30.5 p.p.m.

TABLE II

^{1}H-N.m.r. data at 300 MHz for **8–12** in chloroform-*d* and **13–17** in methanol-d_4

Compound	*Chemical shifts (δ)* *H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*OAc*	*O-*CH_2	CH_2*(2H)*	CH_2 *(mult. H)*	CH_3
8–12	5.16d	5.20dd	5.50dd	4.89dd	4.36m	2.42m(2 H)		2.10, 2.02, 2.01	4.00m (2 H)	1.54[a] m	1.255[b] s	0.86t
13–17	5.09d	3.49dd	3.77[a] t	3.14[a] dd	4.15[d] td	2.84dd	2.37[a] dd		4.085[b,c]	1.62[a] m	1.305[b] s or nm	0.905[b] t
	Coupling constants (Hz) $J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$					
8–12	3.7–3.8	9.5–9.8	9.2–9.4	10.2–10.3								
13–17	3.8	9.3–9.6	8.9–9.2	9.8–9.9	2.6–2.9	9.6–10.2	15.6–15.8					

[a] ±0.01 p.p.m. [b] ±0.015 p.p.m. [c] Center of 2-proton AB-multiplet.

2,3,4-tri-O-acetyl-6-deoxy-α-D-gluco-heptopyranosid]uronates (**8–12**). *A. General procedure.* — To the hexa-*O*-acetyl dicarboxylic acid[12] **5** (70 mg, 0.108 mmol), dissolved in dry 1,2-dichloroethane or benzene (2 mL) in a flame-dried flask, was added oxalyl chloride (0.5 mL). The mixture was heated under N_2 for 95 min at 60° and then evaporated to dryness under reduced pressure, with a drying tube inserted between the evaporator and the pump. Several portions of $ClCH_2CH_2Cl$ were added to, and evaporated from, the solid residue which was then dissolved in fresh $ClCH_2CH_2Cl$ (2 mL) together with an excess (0.55 mmol) of the requisite 1-alkanol. The mixture was stirred for 2 h at 75° and the reaction monitored by t.l.c. (solvent *C*). The solvent was removed and the product chromatographed on a column of SiO_2 (4 g, 230–400 mesh) by use of solvent *E* to elute unreacted alkanol, followed by solvent *D* which produced the desired diester as a homogeneous oil. The yields were 96 (**8**), 86 (**9**), 85 (**10**), and 81% (**11** and **12**).

B. Di-1-octyl ester **8**: R_F 0.30 (solvent *C*), $]\alpha]_D$ +108°, ν_{max} 1745 cm^{-1}, *m/z* (c.i., ether) 429 (100%, 0.5[M −16]$^+$).

Anal. Calc. for $C_{42}H_{66}O_{19}$ (875.0): C, 57.65; H, 7.60. Found: C, 57.79; H, 7.70.

C. Di-1-pentadecyl ester **9**: R_F 0.33 (solvent *B*), $[\alpha]_D$ +85°, ν_{max} 1745 cm^{-1}, *m/z* 1071.5 (2%, M$^+$) and 527.2 (48%, 0.5[M − 16]$^+$).

Anal. Calc. for $C_{56}H_{94}O_{19}$ (1071.4): C, 62.78; H, 8.84. Found: C, 62.63; H, 8.96.

D. Di-1-hexadecyl ester **10**: R_F 0.35 (solvent *B*), $[\alpha]_D$ +85°, ν_{max} 1750 cm^{-1}, *m/z* 541 (21%, 0.5[M − 16]$^+$).

Anal. Calc. for $C_{58}H_{98}O_{19}$ (1099.4): C, 63.36; H, 8.98. Found: C, 63.57; H, 8.88.

E. Di-1-heptadecyl ester **11**: R_F 0.38 (solvent *B*), $[\alpha]_D$ +80°, ν_{max} 1750 cm^{-1}, *m/z* 1127.3 (50%, M$^+$) and 555 (25%, 0.5[M − 16]$^+$).

Anal. Calc. for $C_{60}H_{102}O_{19}$ (1127.5): C, 63.92; H, 9.12. Found: C, 63.77; H, 9.02.

F. Di-1-octadecyl ester **12**: R_F 0.32 (solvent *C*), $[\alpha]_D$ +80°, ν_{max} 1750 cm^{-1}, *m/z* 569.1 (100%, 0.5[M − 16]$^+$).

Anal. Calc. for $C_{62}H_{106}O_{19}$ (1155.5): C, 64.45; H, 9.25. Found: C, 64.58; H, 9.38.

Alkyl [(alkyl 6-deoxy-α-D-gluco-heptopyranosyluronate) 6-deoxy-α-D-gluco-heptopyranosid]uronates (**13–17**). *A. From* **8–12** *by deacetylation. General procedure.* — A solution of the hexaacetyl derivative (65–80 mg, 0.07–0.09 mmol), 5 molar equiv. of the corresponding 1-alkanol (*e.g.*, 80 mg of **12** and 93 mg of 1-octadecanol), and a few crystals of *p*-toluenesulfonic acid in dry 1,2-dichloroethane or benzene (2 mL) was heated at 60°, and the reaction was monitored by t.l.c. After 2 days the t.l.c. pattern ceased to change; solvent *C* revealed some unreacted hexaacetate, some slower-moving, partially deacetylated products, and a strong, immobile spot for fully deactylated product. The latter migrated (R_F 0.40 for **13**, 0.46 for **14–17**) in the more-polar solvent *C*, which also showed the partially deacetylated products as faster-moving spots, and a fast-moving, u.v.-positive by-product. Additional 1-alkanol (5 molar equiv.) and a few more crystals of *p*-TsOH were introduced and heating was continued for 2 days. T.l.c. then indicated absence of the starting hexaacetate (solvent *I*) and presence of an increased proportion of deacetylated diester (solvent *I*). However, partially deacetylated products were still present. The mixture was processed by column chromatography

on SiO_2 (70–230 mesh), using sequentially hexane, 1:4 EtOAc–hexane, solvent *H*, and solvent *G* as eluants. The less-polar solvents eluted 1-alkanol and its acetate, incompletely deacetylated esters, and part of the u.v.-active material, whereas solvent *G* eluted the desired compound, contaminated by traces of the u.v.-active impurity. The fractions containing incompletely deacetylated products were pooled, evaporated, and resubjected to solvolysis for 1 day, as described but with appropriately adjusted proportions of reagents. Chromatography then furnished additional target compound, but some partially acetylated material still remained and was recycled once more in some experiments if this was deemed worthwhile. The combined products obtained as white solids were recrystallized several times from hot MeOH until they were free from the tenaciously adhering, unidentified, u.v.-active contaminant, to give pure **13–17** as follows: **13**, m.p. 131–134° (29 mg, 54%; from 76 mg of **8**); **14**, m.p. 122–124° with sintering at 80° (20 mg, 36%; from 72 mg of **9**); **15**, m.p. 122–123° with sintering at 82° (16 mg, 31%; from 66 mg of **10**); **16**, m.p. 119.5–122° with sintering at 82° (17 mg, 30%; from 72 mg of **11**); and **17** m.p. 114–116° with sintering at 87° (18 mg, 28%; from 80 mg of **12**). The mass-spectral and ^{1}H-n.m.r. data were identical with those of the products obtained by method B.

B. From **4** *and 1-alkyl methanesulfonates. General procedure.* — The mesylates[16] of 1-octanol, 1-pentadecanol, 1-hexadecanol, 1-heptadecanol, and 1-octadecanol were prepared by stirring solutions of the alcohol (~150 mg) and methanesulfonyl chloride (1.2 molar equiv.) in pyridine (1–2 mL) for 1 h at room temperature. Cold water was added to the mixture which, after 10 min, was extracted with ether. The extracts were washed sequentially with 5% HCl, saturated aq. $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated to give oils showing ν_{max} 1465 (CH_2), 1360 (CH_3, S=O), 1175 (S=O), and 945 (S–O); n.m.r. ($CDCl_3$): ^{1}H, δ ~3.0, and ^{13}C, δ ~39 (SO_3Me). Except for the liquid octyl ester[17], the mesylates were obtained as fine needles by crystallization from Me_2CO at −18°, m.p. 51.5–52.5° ($C_{15}H_{31}OMs$), 54–56° ($C_{16}H_{33}OMs$), 59.5–62° ($C_{17}H_{35}OMs$), and 60.5–62° ($C_{18}H_{37}OMs$).

A solution of dicarboxylic acid[12] **4** (22.3 mg, 0.056 mmol) and anydrous K_2CO_3 (8.5 mg, 0.062 mmol) in water (2 mL) was freeze-dried. To the residue was added 1-pentadecyl mesylate (85 mg, 0.28 mmol) and Me_2SO (1.2 mL), and the cloudy mixture was stirred in a closed vial for 1.5 h at 75°, during which it became homogenous. The product **14** (R_F 0.46) was accompanied by faster- and slower-moving by-products (t.l.c. with solvent *I*). The mixture was freeze-dried and then chromatographed on SiO_2 (5 g, 70–230 mesh) by sequential use of solvent *A*, EtOAc, and solvent *F*, to yield **14** (29 mg, 63%; 22 mg after recrystallization from MeOH).

Compounds **13** and **15–17** were obtained analogously in yields of 72, 62, 56, and 68%, respectively, after chromatography, reduced to 63, 53, 47, and 54% after recrystallization from MeOH. (For the preparation of **13**, the liquid mesylate was administered as an ethereal solution, and the ether was allowed to evaporate, in an N_2 atmosphere, prior to the addition of solvent Me_2SO.)

C. Di-1-octyl ester **13**: m.p. 132–134.5°, $[\alpha]_D$ +63°, ν_{max} 3300 and 1730 cm^{-1}; *m/z* 623 (4%, M^+), 605 (13%, $M^+ - H_2O$), 587 (2%, $M^+ - 2H_2O$), 569 (4%, $M^+ - 3H_2O$),

303 (100%, 0.5[M − 16]$^+$), 285 (100%, [0.5(M − 16) − H_2O]$^+$), 267 (47%, [0.5(M − 16) − 2 H_2O]$^+$).

Anal. Calc. for $C_{30}H_{54}O_{13}$ (622.8): C, 57.86; H, 8.74. Found: C, 57.80; H, 8.70.

D. Di-1-pentadecyl ester **14**: m.p. 122.5–124.5° (with sintering at 78°), $[\alpha]_D$ +62°, ν_{max} 3300 and 1730 cm^{-1}; *m/z* 819.1 (8%, M$^+$), 801 (4%, M$^+$ − H_2O), 401.5 (70%, 0.5[M − 16]$^+$), 383 (99%, [0.5(M − 16) − H_2O]$^+$), 365 (21%, [0.5(M − 16) − 2 H_2O]$^+$.

Anal. Calc. for $C_{44}H_{82}O_{13}$ (819.1): C, 64.52; H, 10.09. Found: C, 64.45; H, 10.12.

E. Di-1-hexadecyl ester **15**: m.p. 122–124° (with sintering at 82°), $[\alpha]_D$ +61°, ν_{max} 3300 and 1730 cm^{-1}; *m/z* 415 (12%, 0.5[M − 16]$^+$), 397 (12%, [0.5(M − 16) − H_2O]$^+$).

Anal. Calc. for $C_{46}H_{86}O_{13}$ (847.2): C, 65.22; H, 10.23. Found: C, 64.96; H, 10.14.

F. Di-1-heptadecyl ester **16**: m.p. 119.5–122° (with sintering at 85°), $[\alpha]_D$ +62°, ν_{max} 3300 and 1730 cm^{-1}; *m/z* 429 (18%, 0.5[M − 16]$^+$), 411 (6%, [0.5(M − 16) − H_2O]$^+$).

Anal. Calc. for $C_{48}H_{90}O_{13}$ (875.2): C, 65.87; H, 10.36. Found: C, 65.67; H, 10.23.

G. Di-1-octadecyl ester **17**: m.p. 114–116° (with sintering at 84–87°), $[\alpha]_D$ +61°, ν_{max} 3300 and 1730 cm^{-1}; *m/z* 443.2 (7%, 0.5[M − 16$^+$), 425 (17%, [0.5(M − 16) −H_2O]$^+$), 407 (4%, [0.5(M − 16) − 2 H_2O]$^+$).

Anal. Calc. for $C_{50}H_{94}O_{13}$ (903.3): C, 66.48; H, 10.49. Found: C, 66.68; H, 10.43.

ACKNOWLEDGMENT

This work was supported by the Natural Sciences and Engineering Research Council Canada.

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