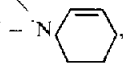
(voucher A-1602) (31 g) afforded 7 mg **6** and 1.5 mg **7**; *Leuocyclus formosus* (voucher 7-14-3) (18 g) gave 15 mg **2a**–**2d** and **3**, 8 mg **8** and 2 mg **9**.

*Tetradeca-, trideca-, dodeca-undeca and deca-*2t,4t-*dienoic isobutyl amide* (**2a**–**2d** *and* **3**). These were obtained as an oily mixture, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3440, 3300, 1660, 1620 (CH=CH)$_2$ CONHR); MS *m/z* (rel. int.): 279.256 (M$^+$, 12) ($C_{18}H_{23}NO$), 265.241 (M$^+$, 2) ($C_{17}H_{31}NO$), 251.225 (M$^+$, 1) ($C_{16}H_{29}NO$), 237.209 (M$^+$, 2) ($C_{15}H_{27}NO$), 223. 191 (M$^+$, 27) ($C_{14}H_{25}NO$).

*Deca-*2t,4t-*dienoic-*2′,3′-*dehydropiperideide* (**5**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1640, 1610, 1005 (CH=CH)$_2$CON ); MS *m/z* (rel. int.): 233.162 (M$^+$, 25) ($C_{15}H_{23}NO$), 151 (M −  , 70), 55 (C_4H_7, 100).

*Tetradeca-*2t,6t,8t,-12c-*tetraen-*10-*ynoic isobutyl amide* (**6**). Colourless gum, UV $\lambda_{max}^{Et_2O}$ nm: 310, 294, 279, 211; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3460, 3320, 1690, 1640 (CH=CHCONHR), 2230 (C≡C), 990 (*trans, trans*-diene); MS *m/z* (rel. int.): 299.225 (M$^+$, 19) ($C_{20}H_{29}NO$), 284 (M − Me, 2), 270 (M − Et, 2), 270 (M − $CHMe_2$, 2), 242 (M − CH_2CHMe_2, 3), 159 (**A***, 100).

*Tetradeca-*2t,6t,8t,12c-*tetraen-*10-*ynoic* 2′,3′-*dehydro pyrrolideide* (**7**). Colourless gum, UV $\lambda_{max}^{Et_2O}$ nm: 311, 294, 281; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1660, 1608 (CH=CHCON), 990 (*trans, trans*-diene); MS *m/z* (rel. int.): 295.194 (M$^+$, 8) ($C_{20}H_{25}NO$), 227 (M −  , 4), 199 (227 − CO, 8), 159 (**A**,* 30), 69 (C_4H_7N, 100).

*Tetradeca-*2t,4t,8c-*trienoic isobutyl amide* (**8**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3440, 3290, 1660, 1620, 1000 [(CH=CH)$_2$ CONHR], 1630 (CH=CH); MS *m/z* (rel. int.): 277.241 (M$^+$, 14) ($C_{18}H_{31}NO$), 262 (M − Me, 4), 248 (M − Et, 2), 205 (M − $NHCH_2CHMe_2$, 38), 167 (M − C_4H_9=$_2$H, 50, McLafferty), 166 (M − C_8H_{15}, 27), 57 ($C_4H_9^+$, 100).

*Tetradeca-*2t,4t,8c,11c-*tetraenoic isobutyl amide* (**9**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 3300, 1675, 1620, 1000 [(CH=CH)$_2$ CONHR], 1605 (CH=CH); MS *m/z* (rel. int.): 275.225 (M$^+$, 5) ($C_{18}H_{29}NO$), 260 (M − Me, 3), 246 (M − Et, 2), 203 (M − $NHCH_2CHMe_2$, 6), 175 (203 − CO, 11), 167 (M − Et=$_3$H, 15, McLafferty), 166 (M − Et=CH_2=CH_2, 11), 67 ($C_5H_7^+$, 100).

*A = C_3H_7=−=$_2CH_2^+$.

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TWO NOVEL HEXASACCHARIDES FROM THE ROOTS OF *ASPARAGUS OFFICINALIS*

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Key Word Index—*Asparagus officinalis*; Liliaceae; novel hexasaccharides; fructo-oligosaccharides; 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_3$sucrose; 1^F(1-β-fructofuranosyl)$_2$-6^G(1-β-fructofuranosyl)$_2$sucrose.

Abstract—Two non-reducing hexasaccharides isolated from the roots of *Asparagus officinalis* were identified as 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_3$sucrose and 1^F(1-β-fructofuranosyl)$_2$-6^G(1-β-fructofuranosyl)$_2$sucrose by examination of the constituent saccharides, GLC analysis of methyl derivatives, and investigation of partial acid hydrolysates and β-fructofuranosidase-catalysed hydrolysis products.

In a series of studies on fructo-oligosaccharides of Liliaceae, my colleagues and I have shown the presence of neokestose in onion bulbs [1], and neokestose and its five related higher oligosaccharides in the roots of asparagus [2, 3]. The present paper deals with the isolation and structural elucidation of hexasaccharides concerned with neokestose from the roots of asparagus.

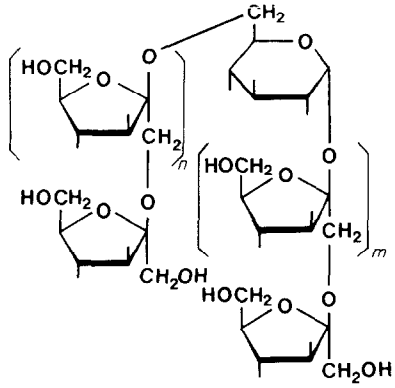
Two fructo-oligosaccharides, saccharides A($[\alpha]_D^{20}$ −14.9°) and B($[\alpha]_D^{20}$ −14.4°), were isolated from an extract of asparagus roots by repeated carbon–Celite column chromatography and preparative paper chromatography. The saccharides were shown to be homogeneous by PC (solvent I) and TLC (solvent III). They were both non-reducing and on hydrolysis with 0.1 M hydrochloric acid or β-fructofuranosidase gave glucose and fructose (PC). The degrees of polymerization were established by measurements of the $[M + Na]^+$ ions (*m/z*, 1013) on FDMS and of the molar ratios (A, 5.86; B, 5.75) of reducing sugar to glucose in acid hydrolysates of the isolated saccharides. These findings showed that saccharides A and B were non-reducing hexasaccharides made up of 1 mol of D-glucopyranose and 5 mol of β-D-fructofuranose.

To clarify the bond structures of the component sugars,

saccharides A and B were permethylated, methanolysed with 1.5% methanolic hydrochloric acid and subjected to GLC. Both of the samples gave six peaks corresponding to methyl-1,3,4,6-tetra-*O*-methyl-D-fructoside [RR_t (R_t of methyl-2,3,4,6-tetra-*O*-methyl-β-D-glucoside = 1), 1.05 and 1.26], methyl-3,4,6-tri-*O*-methyl-D-fructoside (2.68 and 3.98) and methyl-2,3,4-tri-*O*-methyl-D-glucoside (2.53 and 3.57). These results established that saccharides A and B were derivatives of neokestose.

To elucidate the structures of the saccharides, they (20 mg each) were partially hydrolysed with 0.025 M oxalic acid and aliquots of the hydrolysates analysed by PC (solvent II, quadruple development) and TLC (solvent III, triple) as described in a previous paper [10]. Saccharide B gave fructose ($R_{sucrose}$ 1.38 by PC, 1.12 by TLC), glucose (1.27, 1.17), sucrose (1.00, 1.00), neokestose (0.73, 0.78), 1-kestose (0.72, 0.71), 6-β-fructofuranosylglucose (0.86, 0.87) and 6(1-β-fructofuranosyl)$_2$glucose (0.63, 0.63) as well as non-reducing tetra- (R_s 0.53 by PC) and penta- (0.38) saccharides. Saccharide A gave in addition to these compounds 6(1-β-fructofuranosyl)$_3$glucose (0.47 by PC, 0.49 by TLC). The remainders of the hydrolysates were subjected to preparative paper chromatography (solvent II), and the tetra- and pentasaccharides bands were separately extracted from the paper with water, evaporated *in vacuo* and analysed by carbon–Celite column (1.5 × 54 cm) chromatography with 10 and 13% EtOH. It was ascertained from the elution profiles of the saccharides (Fig. 1; pentasaccharides) on carbon–Celite CC that the tetra- and pentasaccharides from saccharide A were 6^G(1-β-fructofuranosyl)$_2$sucrose, 1^F,6^G-di-β-fructofuranosyl sucrose, 6^G(1-β-fructofuranosyl)$_3$sucrose and 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_2$sucrose, and those from saccharide B were 1^F(1-β-fructofuranosyl)$_2$sucrose, 1^F(1-β-fructofuranosyl)$_2$-6^G-β-fructofuranosyl sucrose and the same products without 6^G(1-β-fructofuranosyl)$_3$sucrose as those from saccharide A.

On the basis of the findings presented above, saccharides A and B from the roots of asparagus were shown to be the new hexasaccharides, 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_3$sucrose (**1**) and 1^F(1-β-fructofuranosyl)$_2$-6^G(1-β-fructofuranosyl)$_2$ sucrose (**2**), respectively.



1 Saccharide **A**; $m = 1$, $n = 2$

2 Saccharide **B**; $m = 2$, $n = 1$

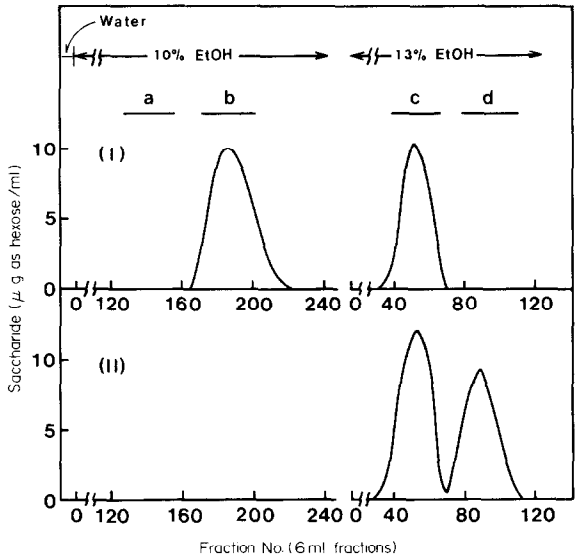


Fig. 1. Carbon–Celite column chromatography of pentasaccharide fractions obtained by partial hydrolyses of saccharides A and B. Pentasaccharide fractions, separated from the partial hydrolysates of saccharides A and B, were chromatographed on carbon–Celite columns (1.5 × 54 cm) by successively eluting with water (0.5 l.), 10% (1.41 l.) and 13% EtOH (1 l.). I and II are chromatograms of pentasaccharides from saccharides A and B, respectively. a–d are the ranges of the fractions containing the reference sugars [10]: (a) 1^F(1-β-fructofuranosyl)$_3$sucrose, (b) 6^G(1-β-fructofuranosyl)$_3$sucrose, (c) 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_2$sucrose, (d) 1^F(1-β-fructofuranosyl)$_2$-6^G-β-fructofuranosylsucrose.

EXPERIMENTAL

Quantitative determination of sugars. Total sugars and reducing sugars were determined by the anthrone [4] and Somogyi Nelson methods [5 7], respectively. Glucose was determined by the use of a commercial Glucostat reagent.

PC and TLC. PC: Solvent I, PrOH–EtOAc–H_2O (6:1:3); solvent II, *n*-BuOH–HOAc–H_2O (4:1:2). TLC (Si gel): solvent III, *n*-BuOH–*iso*PrOH–H_2O (10:5:4). After double to quintuple developments, the chromatograms were sprayed with anisidine phosphate [8] and alkaline $AgNO_3$ [9].

Carbon–Celite CC. For analysing non-reducing tetra- and pentasaccharides, a carbon–Celite CC technique [10] was performed at 15°. A mixture of carbon and Celite-535 (1:1) in a column (1.5 × 54 cm) was washed with HCl and then with H_2O, sugar solution was chromatographed by eluting successively with water, 10%, 13%, and 20% EtOH.

Isolation of hexasaccharides. As described in the previous paper [2], extraction of asparagus roots (4 kg) with hot 70% EtOH (10 l. × 4) containing a small amount of $CaCO_3$ gave an extract, which was concd *in vacuo* to 2 l. and treated with basic $Pb(OAc)_2$ in the usual way. The filtrate was neutralized with 0.5 M NaOH and then concd to yield a soln containing a mixture of sugars (162 g as hexose). The sugar soln was applied to a carbon Celite (1:1) column (7.5 × 72 cm; pre-washed with HCl and then with H_2O) and successively eluted with H_2O (15.0 l.), 10% (15.7 l.), 20% (6.2 l.) and 30% EtOH (10.0 l.). The eluates with 10% EtOH gave three fractions (II, 22.7 g; III, 23.2 g and IV, 1.52 g, in order of elution). Fraction III (20 g) was rechromatographed on a carbon–Celite column (1:1; 5.2 × 80 cm) to afford 11 fractions (total solids, 10.35 g) eluting with 10% EtOH (25.9 l.) and one fraction (7.06 g) with 30% EtOH (3 l.). The latter fraction containing penta-, hexa- and higher saccharides in larger amounts was used in the present study.

This fraction (1 g × 2) was subjected to prep PC with solvent I and a band corresponding to that of hexasaccharides was extracted with H_2O. This extract was concd *in vacuo* and lyophilized to give a white powder (336 mg), which was applied to a carbon–Celite column (1:1; 5.5 × 62 cm; pre-washed with HCl) which, after washing with H_2O (1.0 l.), was eluted successively with 13% (5.0 l.), 15% (5.0 l.) and 17% EtOH. Two fractions eluted with 17% EtOH of volumetric ranges 0.8–2.0 l. and 2.4–4.0 l. were pooled, desalted with Amberlite IR 120B and Amberlite IRA 410, concd and lyophilized to afford two white powders (95 and 110 mg). They were termed saccharide A and saccharide B in order of emergence.

Methylation and methanolysis. Methylation of the isolated saccharides was conducted by the method of Hakomori [11] as described in the previous paper [2], and the methylated saccharides were methanolysed by heating with 1.5% MeOH–HCl at 92° for 5 min. The reaction mixture was treated with Amberlite IR 120B and IRA 410 to remove HCl, and evapd *in vacuo* to dryness.

GLC of methanolysates. The methanolysates were dissolved in a small quantity of MeOH and injected onto a stainless steel column (3 mm × 1 m) packed with 15% butane-1,4-diol succinate polyester on acid-washed Celite. The flow rate of carrier nitrogen gas was 40 ml/min.

Hydrolysis. (1) Partial hydrolysis: the isolated saccharide (20 mg) was dissolved in 0.025 M $(COOH)_2$ (5 ml) and partially hydrolysed by heating at 60° for 15 min. (2) Complete hydrolysis: the isolated saccharide (2 mg) was dissolved in 0.1 M HCl (0.5 ml) and hydrolysed by heating at 100° for 30 min. (3) Enzymatic hydrolysis: the isolated saccharide (2 mg) was hydrolysed by incubating with β-fructofuranosidase (0.2 ml; 0.4 mg of Sigma VI yeast β-fructofuranosidase in 0.2 ml of McIlvaine buffer, pH 5.5) at 30° for 15 hr.

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ANGELOYLCUMAMBRIN-B, AN ANTIMICROBIAL SESQUITERPENE LACTONE FROM *CHRYSANTHEMUM ORNATUM* VAR. *SPONTANEUM*

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Key Word Index—*Chrysanthemum ornatum* var. *spontaneum*; Compositae; angeloylcumambrin-B; guaianolide; new sesquiterpene lactone; microbial growth-inhibitor.

Abstract—Angeloylcumambrin-B, a new antimicrobial guaianolide sesquiterpene lactone, was isolated from *Chrysanthemum ornatum* and the structure was determined by a combination of chemical and physical methods.

In the continuing research for physiologically active sesquiterpene lactones of the Compositae [1–3], we have examined the fresh whole herbs of *Chrysanthemum ornatum* Hemsl var. *spontaneum* (Makino) Kitam. [≡*C. japonense* (Makino) Nakai] gathered in November 1977 in Kagoshima, Japan. In the present paper, we describe the isolation and structure determination of a new antimicrobial guaianolide sesquiterpene lactone, angeloylcumambrin-B (**1**) (0.01%), together with the previously known sesquiterpenoids, cumambrin-A (**2**) (0.05%) [4], cumambrin-B (**3**) (0.0075%) [4], and handelin (**4**) (0.075%) [5].

Angeloylcumambrin-B [(**1**); colourless oil; $[\alpha]_D^{20}$ +100° (c = 0.2, MeOH); UV λ_{max} nm (ε): 211 (13 300); CD: $[\theta]_{223}$ +13 200, $[\theta]_{260}$ −2000] showed the MS molecular ion at m/z 346, in agreement with the molecular formula $C_{20}H_{26}O_5$. The presence of an α-methylene-γ-lactone moiety was confirmed by IR bands ($CHCl_3$) at 1770 and 1670 cm^{-1} and also by the presence in the ^1H NMR spectrum of a characteristic pair of low-field doublets at δ 6.13 (1H, J = 3.0 Hz) and δ 5.47 (1H, J = 2.5 Hz) and in the ^{13}C NMR spectrum of a triplet at

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