SYNTHESIS OF ASCARYLOSE FROM 3,6-DIDEOXY-L-ERYTHRO-HEXOS-2-ULOSE

V. N. Shibaev, V. A. Petrenko,
L. L. Danilov, N. S. Utkina,
M. I. Struchkova, and N. K. Kochetkov

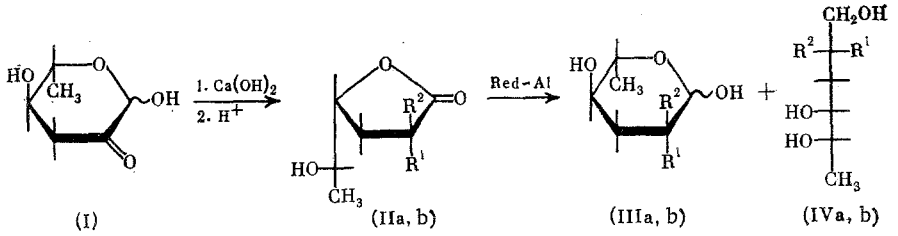
UDC 542.91:547.455.566

3,6-Dideoxy-L-arabino-hexose (ascarylose) (IIIa) is a component of the egg glycolipids of the parasitic worm *Parascaris equorum* and of the lipopolysaccharides of Gram-negative bacteria. The structure of this monosaccharide has been established on the basis of its dehydration products [1] and by comparison with a synthetic enantiomer (tivelose) [2]. The first synthesis of ascarylose has been performed recently [3].

The preparation of this monosaccharide and its derivatives is of considerable interest for immunochemical research and for studying the biosynthesis of natural polymers containing it. Ascarylose is an analog of L-rhamnose in which the OH group attached to C_3 is replaced by an H atom. The object of the present work has been to prepare this analog in connection with our investigations into the specificity of enzymes involved in the biosynthesis of *Salmonella* O antigen using synthetic analogs of thymidine diphosphate rhamnose [4].

Ascarylose was synthesized by reaction scheme 1.

Scheme 1



(I) (IIa, b) (IIIa, b) (IVa, b)

R^1=OH, R^2=H (IIa), (IIIa), (IVa); R^1=H, R^2=OH, (IIb), (IIIb), (IVb). Red-Al=$NaAlH_2(OCH_2CH_2OCH_3)_2$

The starting 3,6-dideoxy-L-erythro-hexos-2-ulose (I) was prepared [5] by dehydration of L-rhamnose by reacting the latter with benzoylhydrazine and p-toluidine followed by reaction with benzaldehyde. The dehydration of L-rhamnose was carried out under the conditions recommended for the analogous reaction of D-glucose [6], which considerably increased the yield of the bisbenzoylhydrazone of (I) in comparison with [5] (see Experimental section). The syrupy ulose (I) was characterized by converting it into the crystalline bis-2,4-dinitrophenylhydrazone in a yield of 90%.

The rearrangement of 3-deoxyhexosuloses into 3-deoxyaldonic acids under the influence of alkaline reagents is well known.

Treatment of (I) with a saturated $Ca(OH)_2$ solution (1 h at ~20°C) produces a complex mixture of products. Acidification of this mixture and chromatographic separation on silica gel gives a mixture of 3,6-dideoxyaldonic acid lactones (IIa) and (IIb) in a total yield of 13%. Increasing the reaction time to 18 h, as recommended for hexose and pentose derivatives in [6], or carrying out the rearrangement in an argon atmosphere, has no significant effect on the yield of the products. The isomeric lactones were separated by preparative paper chromatography; their ratio in the mixture is 3:2.

The structure of (IIa) and (IIb) follows unequivocally from their combined physicochemical data. Their IR spectrum contains a strong band at 1780 cm^{-1}, which is characteristic

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR, Moscow.
Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 1, pp. 158-163, January, 1980. Original article submitted November 13, 1978.

0568-5230/80/2901-0143$07.50

TABLE 1. Chemical Shifts in the ^{13}C NMR Spectra of Aldonic Acid Lactones

Compound	δ, ppm					
	C^1	C^2	C^3	C^4	C^5	C^6
(IIa)	180,2	68,8	31,2	81,3	67,9	17,2
(IIb)	180,6	68,3	30,5	82,2	67,5	17,9
(V)	178,1	72,3	70,7	84,2	65,0	20,2

of carbonyl stretching in γ-lactones. Their PMR spectra contain readily identifiable signals indicating the presence of CH_3 and CH_2 groups, and the signals from the CHO protons at C_5 and C_4 can be discriminated by double resonance.

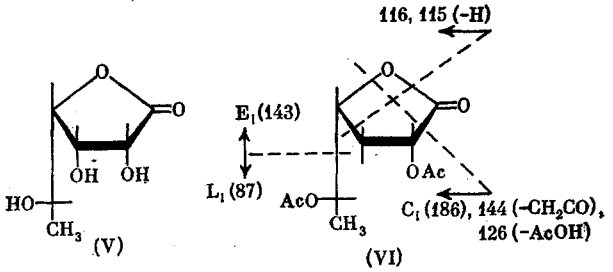
Substantial information about the structure of the compounds was obtained by analyzing their ^{13}C NMR spectra and comparing them with the spectrum of rhamnonic acid γ-lactone (V). As can be seen from Table 1, the spectrum confirms the presence of CO, CH_3, CH_2, and three CHO groups and unequivocally indicates the five-membered cyclic form of (IIa) and (IIb) (characteristic signal in the 81-85 ppm region) [7, 8].

The assignment of the ^{13}C signals of the CHOH groups in the spectrum of (V) is based on the fact that in the spectra of alkyl furanosides, particularly α-methyl-D-mannofuranoside, in which there are the same interactions between the substituents in the furanose ring as in (V), the relative positions of the signals correspond to the sequence C_2, C_3, C_5 (C_5 being at highest field) [8], and the introduction of a deoxy unit at C_6 shifts the C_5 signal to even higher field. On passing from (V) to (IIa) and (IIb), the introduction of a deoxy unit at C_3 must produce a high-field shift in the signals of the adjacent carbon atoms C_2 and C_4; this allows us to make an unequivocal assignment of the signals in the spectra being analyzed.

Isomers (IIa) and (IIb) are present in the mixture in different amounts, and the differences in the intensity of the signals makes it possible to determine to which of the substances they belong. Furthermore, we can form certain conclusions regarding the configuration of the isomers by careful analysis of the differences in the chemical shifts of the C_2, C_3, and C_4 signals. According to [8], the chemical shifts of the ring C atoms in pentofuranoses can be predicted quite accurately on the basis of an additive scheme taking into account the interaction of cis substituents. In particular, the interaction of the cis substituents at C_2 and C_4 will result in a small low-field shift in the C_2 and C_3 signals and a small high-field shift in the C_4 signal (compared with the compound having a trans arrangement of substituents at C_2 and C_4). Just such differences are observed in the spectra of the isomeric lactones: the L-arabino configuration (IIa) (with 2,4-cis substituents) can be assigned to the predominant isomer in the mixture, and the L-ribo configuration (IIb) (with 2,4-trans substituents) can be assigned to the other isomer.

The five-membered ring structure of lactone (IIa) is also confirmed by the mass spectrum of its diacetate (VI): one of the principal routes for primary fragmentation of the molecule ion of (VI) (see scheme 2) comprises rupture of the C_4—C_5 bond to form an ion L_1 and a series of ions E (using the nomenclature proposed in [9]); this dissociation route is common to furanose derivatives [9]. This route and two other routes for primary fragmentation of the molecule ion (see scheme 2) are known for simpler lactones [10].

Scheme 2



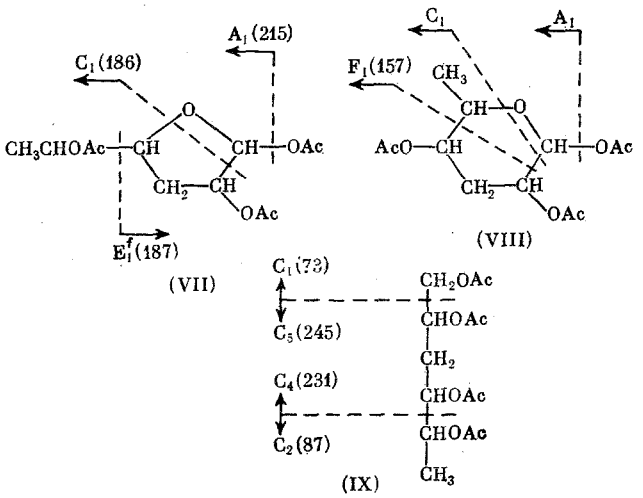
Confirmation of the configuration of the chiral center at C_2 in lactones (IIa) and (IIb) was obtained on the basis of the optical rotation of the products. According to the data in

[11], 3-deoxy-D-ribo-hexonic acid γ-lactone has an appreciably greater rotation ($[M]_D$ = +42.4°) than the 3-deoxy-D-arabino lactone (+9.4°); accordingly, the L-ribo enantiomer should have a more negative molecular rotation. Bearing in mind that the molecular rotation increases by ∿30° on passing from L-series hexoses to 6-deoxyhexoses [12], we can expect a $[M]_D$ value of +21° for the 3,6-dideoxy derivative with the L-arabino configuration (IIa) and −12° for the L-ribo derivative (IIb). The experimentally observed $[M]_D$ values for (IIa) and (IIb) (+17.4 and −4.5°, respectively) are in reasonable agreement with the calculated values. As in the case of other 3-deoxyaldonic acid γ-lactones [6], the isomer with a 2,4-threo arrangement of substituents (IIa) is formed in higher yield and has a lower paper-chromatographic mobility than the 2,4-erythro isomer (IIb).

A number of methods have been suggested for reducing aldonic acid lactones to aldoses (see [13] and references therein). In particular, good results have been obtained using lithium aluminum hydride [14], and sodium bis[2-methoxyethoxy)aluminum hydride, which has a comparable reducing power but is more convenient to use, has been used successfully for preparing 4,6-dideoxyaldoses from 4,6-dideoxyaldonic acid lactones [15]. We studied the reduction of a mixture of lactones (IIa) and (IIb) under the influence of this reagent.

The best results were obtained when the reaction was carried out in THF—dioxane at −22°C. The starting lactones disappeared completely in 2 h and the reaction products contained 69% of aldoses (IIIa, b) and 31% of polyols (IVa, b). To establish the structure of the products, the product mixture from one experiment was acetylated and then subjected to combined chromatographic and mass spectroscopic analysis. The results obtained clearly indicate the presence of three types of compounds in the analysis mixture, viz., 3,6-dideoxyhexofuranose triacetates (VII), 3,6-dideoxyhexopyranose triacetates (VIII), and 3,6-dideoxyhexitol tetraacetates (IX), which can be reliably identified by characteristic fragmentation in the mass spectrum [9] (scheme 3)

Scheme 3



Preparative separation of the aldoses and polyols was effected by chromatography on silica gel, and the individual aldoses were isolated by chromatography in borate buffer using a carbohydrate analyzer. This gave ascarylose (IIIa) in a yield of 35% based on the mixture of (IIa) and (IIb). The product is not distinguishable from an authentic sample of the enantiomeric 3,6-dideoxy sugar tivelose by paper chromatography or in the carbohydrate analyzer; its optical rotation corresponds to literature data [2, 3]. Reduction of (IIIa) with $NaBH_4$ yields the crystalline polyol (IVa), which has the same melting point as that described in the literature. The second 3,6-dideoxy sugar (IIIb) (10% yield) has an optical rotation which is close in absolute magnitude and opposite in sign to that of 3,6-dideoxy-D-ribo-hexose (paratose) [2].

EXPERIMENTAL

Adsorption TLC was performed on silica gel KSK plates using the following solvent systems: A) $CHCl_3/CH_3OH$ (5:1); B) $CHCl_3/CH_3OH$ (9:1). Silica gel L 40/100 μ (Czech) was used for column chromatography. Partition chromatography was performed with Watman 3 mm paper using the following solvent systems: C) $C_5H_5N/n\text{-}C_4H_9OH/H_2O$ (4:6:3); D) methyl ethyl

ketone saturated with water. Lactones were detected on the paper chromatograms by the hydroxamic acid test, reducing sugars were detected by means of aniline acid phthalate, and nonreducing carbohydrate derivatives were detected by the method described in [16]. Anion-exchange chromatography was performed with DA × 4 anion exchanger (Durrum, USA) using a 71 100A liquid chromatograph (Czech) with a 10 × 0.6 cm analytical column (borate buffer supply rate 20 ml/h, detecting reagent 0.1% orcine solution in 85% H_2SO_4). Optical rotation was measured with a Perkin–Elmer 141 automatic polarimeter. Melting points were determined with a Kofler unit. The ^{13}C NMR spectra were measured with a WP-60 spectrometer (15.08 MHz) with complete suppression of proton coupling. The PMR spectra were measured with a Tesla BS 467 spectrometer (100 MHz). The solvents used were CD_3OD and D_2O, and the internal standard was HMDS. The IR spectra were obtained with a Specord IR-75 spectrophotometer using KBr pellets. The mass spectra were recorded with a Varian MAT 111 chromatographic mass spectrometer (column packed with 10% OV-1 on Chromosorb W 60/80) at an initial temperature of 120°C (programmed at 8 deg/min). The samples for recording mass spectra were acetylated with Ac_2O in pyridine at ∿20°C for 5 h. In the mass spectra quoted, we list all peaks with m/e > 43 and with an intensity >10% of that of the m/e = 43 peak, and also certain characteristic peaks of lower intensity.

3,6-Dideoxy-L-erythro-hexos-2-ulose Bisbenzoylhydrazone. A solution of 2 g L-rhamnose in 72 ml ethanol, 1.6 ml AcOH, and 8 ml water was treated with 0.8 g p-toluidine, boiled for 30 min, treated with 2.6 g benzoylhydrazine, and boiled for 7 h. The reaction mixture was cooled, concentrated to 30 ml, and left to stand for 16 h. The crystals were filtered off and washed with ethanol and ether to give 2.15 g (56%) of product, R_f = 0.8 (B), mp 234°C (from ethanol); cf. [5].

3,6-Dideoxy-L-erythro-hexos-2-ulose (I). Compound (I) was prepared from 3,6-dideoxy-L-erythro-hexos-2-ulose bisbenzoylhydrazone as in [5]; yield 97%, R_f = 0.37 (B).

3,6-Dideoxy-L-erythro-hexos-2-ulose Bis-2,4-dinitrophenylhydrazone. This was prepared as in [5], yield 90%, R_f = 0.91 (C), mp 269°C (from ethanol), cf. [5].

3,6-Dideoxy-L-arabino- and 3,6-Dideoxy-L-ribo-hexonic Acid γ-Lactones (IIa) and (IIb). Ulose (I) (0.9 g, 6 mmole) was added to 540 ml of a saturated $Ca(OH)_2$ solution. The mixture was left to stand for 1 h at ∿20°C and then passed through a column (50 × 2.5 cm) of Dowex 50W × 1 (H^+ form). The eluate was evaporated and poured onto a column (20 × 2 cm) of silica gel. The column was washed with 50 ml of $CCl_4/CHCl_3$ (1:1), 50 ml of $CCl_4/CHCl_3$ (1:4), and 100 ml of $CHCl_3$. The lactones (IIa, b) were eluted with 150 ml of $CHCl_3$/MeOH (20:1). The yield of the mixture of (IIa) and (IIb) was 0.111 g (0.8 mmole, 13%), R_f = 0.53 (A), 0.50 (B). PMR spectrum (δ, ppm): 1.10 and 1.12 d (CH_3, $J_{5,6}$ = 6 Hz), 1.9-2.5 m (CH_2), 3.92 m (H^5), 4.17 m (H^2), 4.44 d.t (H^4).

A 20 mg portion of the mixture of (IIa) and (IIb) was subjected to preparative paper chromatography with solvent D, giving 11.4 mg of (IIa), R_f = 0.89 (D), $[\alpha]_D^{20}$ = +11.9° (c = 1, H_2O), and 7.6 mg of (IIb), R_f = 0.95 (D), $[\alpha]_D^{20}$ = −3.1° (c = 1, H_2O). Mass spectrum of (IIa) acetate, m/e: 230 (6, M^+), 188 (4), 186 (7), 144 (25), 143 (97), 128 (10), 127 (10), 126 (84), 116 (12), 115 (99), 110 (32), 103 (80), 101 (12), 100 (16), 87 (40), 85 (15), 84 (99), 83 (100), 82 (17), 71 (17), 69 (10), 61 (36), 57 (22), 56 (42), 55 (99), 45 (22), 44 (50), 43 (100).

Reduction of Lactone Mixture (IIa, b). A mixture of 2 ml $NaAlH_2(OCH_2CH_2OCH_3)_2$ (70% solution in toluene) and 6 ml dry dioxane was cooled to −70°C, treated with a solution of 111 mg of lactone mixture (IIa, b) in 4 ml dry THF, and stirred at −23°C for 2 h. The excess reducing agent was decomposed with THF/water (4:1) with cooling. Cations were removed by treating the reaction mixture with 1 g of Dowex 50W × 1 (H^+ form). The aqueous filtrate was evaporated down to a syrup and poured onto a column (30 × 2 cm) of silica gel. The column was washed with 100 ml portions of $CHCl_3$/MeOH mixtures containing 10, 20, 30, and 40 vol.% MeOH. The fractions containing aldoses (IIIa, b) (eluted with 20% MeOH in $CHCl_3$) were combined and evaporated to dryness, giving 50.8 mg (45.3%) of (IIIa, b), R_f = 0.37 (A). The yield of mixed polyols (IVa, b) (eluted with 40% MeOH in $CHCl_3$) was 23 mg (20.4%), R_f = 0.16 (A).

The aldoses (IIIa) and (IIIb) were separated by anion-exchange chromatography on a column (60 × 0.8 cm) of anion exchanger DA × 4. The flow rate of borate buffer (0.2 M, pH 8.75) was 60 ml/h at 55°C. The monosaccharide-containing fractions were desalinated by treating with cation exchanger KU-2 (H^+ form) and evaporated with MeOH. The yield of ascarylose (IIIa) was 34.5 mg, R_{Rha} = 4.0 (D), $[\alpha]_D^{20}$ = −26° (c = 18.9, MeOH), cf. [2]. The

yield of 3,6-dideoxy-L-ribo-hexose (IIIb) was 12 mg, $R_{Rha} = 3.4$ (D), $[\alpha]_D^{20} = -11°$ (c = 6.1, MeOH).

Chromatographic Mass-Spectrometric Analysis of Products Obtained by Reducing Lactone Mixture (IIa, b). The lactone mixture (IIa, b) was reduced, decationized and acetylated as described above.

Mass spectrum of (VIII), *m/e*: 232(30), 215(36), 155(7), 154(13), 145(17), 144(16), 143(68), 129(47), 126(16), 113(56), 112(60), 103(70), 102(50), 101(16), 100(54), 97(28), 95(25), 94(12), 89(10), 86(19), 85(36), 84(91), 83(100), 81(12), 71(11), 69(15), 68(57), 61(23), 60(25), 58(30), 57(48), 56(26), 55(62), 45(20), 44(38), 43(100).
Mass spectrum of (VII), *m/e*: 232(8), 215(24), 187(22), 155(4), 145(15), 143(15), 129(18), 127(54), 113(32), 112(26), 103(32), 100(10), 97(14), 95(11), 85(54), 84(25), 83(95), 68(22), 61(10), 60(12), 58(12), 57(20), 56(10), 55(26), 45(14), 44(22), 43(100).
Mass spectrum of (IX), *m/e*: 245(25), 232(10), 231(76), 185(7), 171(22), 157(16), 156(67), 145(15), 143(81), 130(27), 129(100), 115(17), 114(25), 113(17), 112(30), 103(99), 101(20), 97(60), 96(100), 95(27), 87(73), 86(17), 84(32), 83(98), 81(40), 73(11), 71(27), 70(58), 69(100), 61(26), 60(16), 58(10), 57(41), 55(22), 45(21), 44(40), 43(100).

Ascarylitol (IVa). A 3 mg portion of ascarylose (IIIa) was dissolved in 1 ml water and 3 mg of $NaBH_4$ was added. The mixture was kept at ~20°C for 18 h. The excess $NaBH_4$ was decomposed with AcOH (pH 5-6), cations were removed by treating with Dowex 50W × 1 (H^+ form), and the aqueous filtrate was evaporated with methanol, giving 2.4 g (80%) of (IVa), $R_f = 0.16$ (A), $R_{Rha} = 1.1$ (C), mp 110°C (from acetone), cf. [2].

CONCLUSIONS

1. A novel synthesis of 3,6-dideoxy-L-arabino-hexose from L-rhamnose has been performed.

2. The γ-lactones of 3,6-dideoxy-L-arabino- and L-ribo-hexonic acids have been prepared and the ^{13}C NMR spectra of aldonic acid γ-lactones have been analyzed.

LITERATURE CITED

1. C. Fouquey, J. Polonsky, and E. Lederer, Bull. Soc. Chim. Belg., 40, 315 (1957).
2. C. Fouquey, J. Polonsky, and E. Lederer, Bull. Soc. Chim. France, 803 (1959).
3. J.-C. Floreut, C. Monneret, and Qui Khuong-Huu, Carbohyd. Res., 56, 301 (1977).
4. V. N. Shibaev, G. I. Eliseeva, Yu. Yu. Kusov, V. A. Petrenko, S. S. Mishchenko, and N. K. Kochetkov, Izv. Akad. Nauk SSSR, Ser. Khim., 2584 (1976); V. N. Shibaev, Yu. Yu. Kusov, V. A. Petrenko, and N. K. Kochetkov, Izv. Akad. Nauk SSSR, Ser. Khim., 2588 (1976); V. N. Shibaev, Yu. Yu. Kusov, V. A. Petrenko, T. N. Druzhinina, and N. K. Kochetkov, Bioorg. Khim., 4, 249 (1978).
5. H. E. Khadem, D. Horton, M. M. Meshreki, and M. Nashed, Carbohyd. Res., 17, 183 (1971); H. El Khadem and D. Horton, Carbohyd. Res., 22, 381 (1972).
6. Š. Kučar, J. Zamočky, and Š. Bauer, Coll. Czech. Chem. Commun., 40, 457 (1975).
7. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
8. R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, Can. J. Chem., 53, 1424 (1975).
9. N. K. Kochetkov and O. S. Chizhov, Methods of Carbohydrate Research [in Russian], Mir (1975), p. 409.
10. L. Friedman and F. A. Long, J. Am. Chem. Soc., 75, 2832 (1953); W. H. McFadden, E. A. Day, and M. J. Diamond, Anal. Chem., 37, 89 (1965); E. Honkanen, T. Moisio, and P. Karvonen, Acta. Chem. Scand., 19, 370 (1965).
11. H. B. Wood and H. G. Fletcher, J. Org. Chem., 26, 1969 (1961).
12. D. H. Whiffen, Chem. Ind., 964 (1956).
13. S. S. Bhattacharjee, J. A. Schwarez, and A. S. Perlin, Carbohyd. Res., 42, 259 (1975).
14. J. Nemec and J. Jarý, Coll. Czech. Chem. Commun., 34, 1611 (1969).
15. K. Kefurt, Z. Kefurtova, and J. Jarý, Coll. Czech. Chem. Commun., 41, 1791 (1976).
16. A. I. Usov and M. A. Rekhter, Zh. Obshch. Khim., 39, 912 (1969).