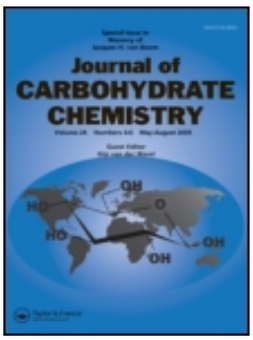

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ASCOPYRONE P: CHEMICAL SYNTHESIS FROM D-GLUCOSE

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ABSTRACT

The pyranone, 1,5-anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose (**1**) (ascopyrone P), has been synthesised in eight steps from D-glucose. The key steps were deacetylation of 3,6-di-*O*-acetyl-1,5-anhydro-D-*glycero*-hex-3-en-2-ulose (**8**) to give isomers and hydrates of 1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulose (**9**). Isomerisation of this mixture afforded 1,5-anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose (**1**) (ascopyrone P) in a moderate yield.

Key Words: Ascopyrone P; Ascopyrone T; Antioxidant

INTRODUCTION

1,5-Anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose (**1**) has as early as 1978[1] been identified as a pyrolysis product of cellulose and characterised by X-ray diffraction and NMR spectroscopy.[2] Recently, the enone **1** has also been identified as a degradation product from α-1,4-glucans in Ascomycetes, when subjected to 'activating' plasmolytic treatments.[3] Several Ascomycetes exhibited an enzymatic activity, which degrades α-

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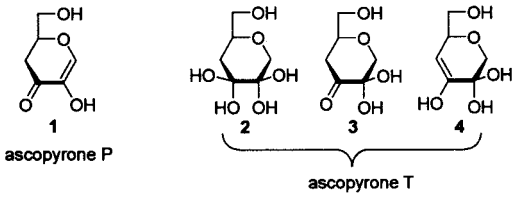


Figure 1. Ascopyrone P and T.

1,4-glucans to 1,5-anhydro-D-fructose, and further into the enone **1** in Pezizales (enone **1** named ascopyrone P) or to a mixture of three hydrated forms of **1** in Tuberales (named ascopyrone T) (Figure 1).

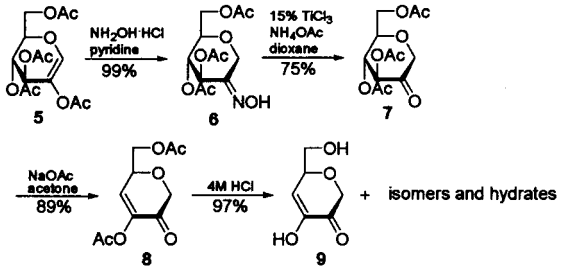
Degradation products from α-1,4-glucans, such as starch, are easily available chiral starting materials from renewable resources and therefore of interest for industrial uses. The lyase responsible for the degradation of α-1,4-glucans to 1,5-anhydro-D-fructose has been cloned and expressed in *Aspergillus niger*,[4,5] whereas the dehydratase responsible for the dehydration of 1,5-anhydro-D-fructose to **1** in Pezizales is not easily available at the present. Initial experiments have shown that **1** possesses some antioxidative properties.[6] Hence, we now wish to report on the chemical synthesis of ascopyrone P and investigate these properties.

RESULTS AND DISCUSSION

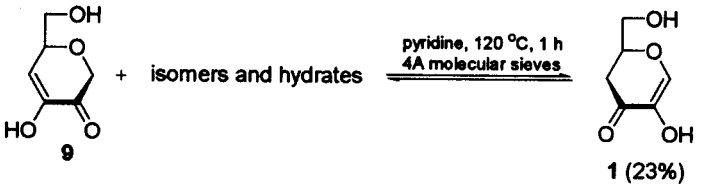
Synthesis of Ascopyrone P

The synthesis and chemistry of unprotected 1,5-anhydro-4-deoxyhexo-2,3-diuloses and similar compounds have been scarcely studied. Several derivatives, such as acylated 1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulose,[7–9] acylated ascopyrone P,[1] benzoylated ''Herzgift-Methylreduktinsäure,''[10] as well as oximes[11,12] and osazones of 1,5-anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose[13,14] have been prepared, but in no cases the unprotected compounds.

We have investigated two approaches for the synthesis of ascopyrone P (**1**), using D-glucose as starting material (Schemes 1–3). In the first approach (Schemes 1 and 2)



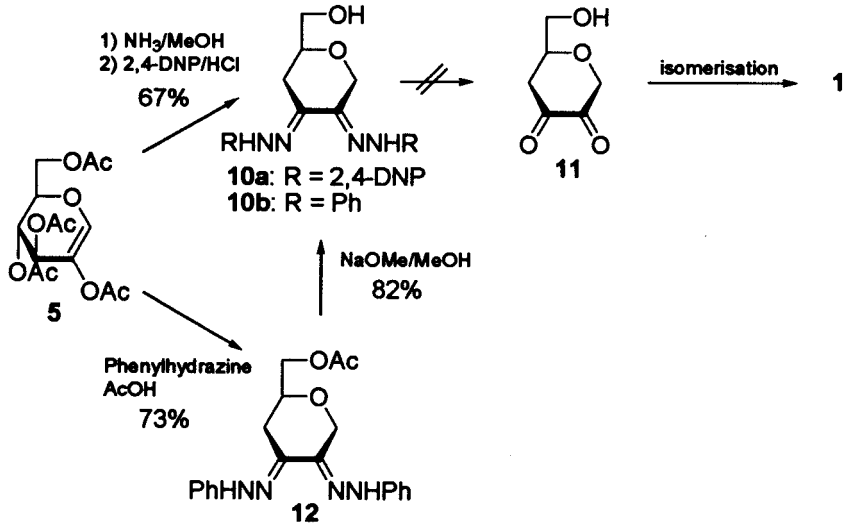
Scheme 1. Synthesis of ascopyrone T.



Scheme 2. Isomerisation to ascopyrone P.

the key intermediate was 3,6-di-*O*-acetyl-1,5-anhydro-D-*glycero*-hex-3-en-2-ulose (**8**), which upon deacetylation, followed by isomerisation, should afford ascopyrone P.

2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (**5**)[15,16] was treated with hydroxylamine in pyridine to afford the known crystalline oxime **6**[17] in good yield (61%). Subsequent reductive deoximination with 15% $TiCl_3$ and NH_4OAc as a buffer, then afforded 3,4,6-tri-*O*-acetyl-1,5-anhydro-D-fructose (**7**).[14] Ketone **7** readily eliminated acetic acid upon treatment with NaOAc in acetone to yield 3,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulose (**8**)[7] which was isolated by column chromatography in an excellent yield (89%). Neither compound **7** nor **8** were stable for more than a few days: acetylated 1,5-anhydro-D-fructose **7** eliminated acetic acid to afford the enone **8**, which decomposed to unidentified products. To prevent elimination and rearrangement reactions from deacetylation of **8**, aqueous acid was used for deprotection, resulting in hydration of the liberated carbonyl moiety. We were pleased to find that the deacetylation of 3,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulose (**8**) in aqueous HCl, gave the enone **9** together with several isomeric forms and hydrates, in a quantitative yield. ^{13}C NMR spectroscopy of the



Scheme 3. The osazone approach.

isolated product showed the dihydrate **2** as the main product. Ascopyrone P (**1**) was not detected.

Since **1** has been reported to be stable in aqueous solution, whereas the other isomers are hydrated,[3] we expected ascopyrone P to be the main product from isomerisation of **9** and its isomers in anhydrous base. Initial experiments to isomerise **9** in pyridine showed the presence of ascopyrone P (**1**), although in low yield. After optimisation of the reaction by varying the concentration of **9**, temperature and reaction-time, **1** could be crystallised from the concentrated reaction mixture in 18% yield. Column chromatography of the mother liquor increased the total yield to 23% (51% based on recovered starting material).

HPLC and TLC showed ascopyrone P (**1**) to be stable in aqueous solution at pH 7, but unstable in acetone, EtOAc and pyridine at ambient temperature, due to isomerisation. Because of that **1** was not recrystallised.

The key intermediates in the second approach were osazones of ascopyrone P, which could be synthesised in a few steps from the acetylated 2-hydroxy-D-glucal **5**. The osazones are attractive precursors for **1** since deprotection should afford the α-diketone **11** (Scheme 3), thus reducing the length of the total synthesis. The phenyl- and 2,4-dinitrophenylosazone of ascopyrone P were prepared according to literature procedures.[13,14,18]

Deacetylation of **5** was performed with ammonia in methanol to afford an oily intermediate, which was treated with excess 2,4-dinitrophenylhydrazine hydrochloride (2,4-DNP/HCl) to give 1,5-anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose 2,4-dinitrophenylosazone (**10a**) in 67% yield.[14,18]

Reaction of **5** with phenylhydrazine in acetic acid yielded 6-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose phenylosazone (**12**) in a good yield and subsequent deacetylation with NaOMe/MeOH gave 1,5-anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose phenylosazone (**10b**) in an excellent yield. Both the acetylated osazones, **12** and **10b**, are strongly coloured compounds and therefore the measured optical rotations are not accurate.

Liberation of the dicarbonyl functions from the osazones **10a** and **10b** turned out to be difficult. All attempts to deprotect the osazones with benzaldehyde/H^+,[19] acetone/H^+ [20] and dimethoxyethane/$TiCl_3$[21] failed, due to the high stability of the osazones **10a** and **10b**. This approach was not further pursued.

Antioxidant Activity of Ascopyrone P

Ascopyrone P (**1**) contains a conjugated keto-enol structure, in analogy with common antioxidants such as butylhydroxyanisole (BHA), dibutylhydroxytoluene (BHT), ascorbic acid and kojic acid, and might therefore have an antioxidant activity. This hypothesis was supported by several preliminary experiments: **1** reduces decoloration of β-carotene when exposed to oxygen and oxidative intermediates of linoleic acid, delays oxidation of linoleic acid and functions as a food antioxidant in mayonnaise and yogurt salad dressing.[6] Since **1** is stable for weeks in aqueous solution, we were able to investigate the antioxidant activity in an aqueous system. The oxidation of linolenic acid was followed by monitoring the formation of the oxidation products, lipid hydroperoxide, malonaldehyde and 4-hydroxynonenal. In the presence of **1**, 160 ppm,

the formation of lipid hydroperoxide was reduced (47%) as well as the formation of malonaldehyde and 4-hydroxynonenal (59% reduction), compared to experiments performed without addition of ascopyrone P (**1**).

CONCLUSIONS

Inspired by the initial promising result of ascopyrone P as an antioxidant, we have successfully developed a chemical synthesis of **1**. Starting from D-glucose we have prepared sufficient amounts of ascopyrone P for further thorough testing of these properties. The oxidation experiments clearly show that **1** has an antioxidant activity, which will be further investigated by addition of **1** to food.

EXPERIMENTAL

General procedures. Melting points were determined with a melting point apparatus (Büchi 510) and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at the Department of Organic Chemistry, Technical University of Denmark. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Gemini 200 MHz instrument at ambient temperature (Danisco) and a Bruker AC 300 MHz at ambient temperature (Department of Organic Chemistry, Technical University of Denmark). For NMR spectra the solvent peak was used as a reference.[22] The microanalyses were carried out at the Chemical Laboratory II, University of Copenhagen. The progress of all reactions was monitored by thin-layer chromatography using aluminium sheets precoated with silica gel 60 F_{254} to a thickness of 0.2 mm. Compounds were detected with UV light (254 nm) and/or by spraying the sheets with a solution of 1.5% ammoniummolybdonate, 1% cerium sulfate and 10% sulfuric acid, followed by heating. Column chromatography was conducted under pressure (2 bar) with silica gel (0.043–0.063 mm). LC-MS was performed on a Hewlett Packard 1100 series with an APCI detector.

3,4,6-Tri-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-2-ulose oxime (6).[17] To a solution of 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (**5**)[15,16] (7.90 g, 23.9 mmol) in dry pyridine (40 mL, 496 mmol), $HONH_2$·HCl (5.85 g, 84.2 mmol) was added and the mixture was stirred for 24 h. The reaction mixture was concentrated and dissolved in $CHCl_3$ (300 mL). The organic phase was washed with 1 M HCl (75 mL), saturated aqueous $NaHCO_3$ (75 mL) and H_2O (75 mL), dried ($MgSO_4$) and concentrated to a syrup of **6** (7.19 g, 99%). Upon addition of a small volume of EtOH the product crystallised (4.43 g, 61%, mp 86–89°C). Two recrystallisations from toluene afforded an analytical sample: mp 90–92°C; $[\alpha]_D - 39.4°$ (*c* 1.3, $CHCl_3$) [Lit.[17] mp 89–90°C, $[\alpha]_D - 39.0$ (*c* 0.4, $CHCl_3$)]. ^{1}H NMR (DMSO-d_6 at 2.50, 300 MHz) δ 1.99 (s, 3H, OCOCH_3) 2.02 (s, 3H, OCOCH_3), 2.03 (s, 3H, OCOCH_3), 3.87 (ddd, $J_{5,6} = 3.0$, $J_{5,6'} = 5.5$, $J_{4,5} = 8.5$, 1H, H-5), 4.03 (d, $J_{1,1'} = 15.0$, 1H, H-1), 4.05 (dd, $J_{6,6'} = 12.0$, 1H, H-6), 4.12 (dd, 1H, H-6′), 4.88 (d, 1H, H-1′), 4.93 (dd, $J_{3,4} = 8.0$, 1H, H-4), 5.54 (d, 1H, H-3), 11.42 (s, 1H, NOH). ^{13}C NMR (DMSO-d_6 at 39.5, 50.3 MHz) δ 20.6 (3 × OCO*C*H_3), 60.9 (C-1), 62.5 (C-6), 69.3 (C-4), 70.5 (C-3), 74.9 (C-5), 148.9 (C-2), 169.3–170.2 (3 × O*C*OCH$_3$).

Anal. Calcd for $C_{12}H_{17}NO_8$: C, 47.53; H, 5.65; N, 4.62. Found: C, 47.57; H, 5.56; N, 4.50.

3,4,6-Tri-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-2-ulose (7).[14,23] 3,4,6-Tri-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-2-ulose oxime (**6**) (5.00 g, 16.5 mmol) was dissolved in dioxane (100 mL) and NH_4OAc (13.0 g, 169 mmol) was added. The mixture was cooled on ice, 15% $TiCl_3$ (44 mL, 54 mmol) was added and the reaction mixture was stirred at rt for 3 h. The mixture was extracted with $CHCl_3$ (5×30 mL) and the combined organic phase was washed with saturated aqueous $NaHCO_3$ ($70 + 50$ mL). The combined aqueous phase was extracted with $CHCl_3$ (30 mL) and the combined organic phase was washed with H_2O (30 mL). The organic phase was dried ($MgSO_4$) and concentrated to a syrup of **7** (3.54 g, 75%). Upon addition of Et_2O, the product crystallises (1.29 g, 27%, mp 81–85°C). Two recrystallisations from Et_2O afforded an analytical sample: mp 93–94°C; $[\alpha]_D - 7.2$ (*c* 1.5, $CHCl_3$) [Lit.[23] mp 86–88°C, $[\alpha]_D - 10$ (*c* 0.5, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$ at 7.27, 300 MHz) δ 2.08 (s, 3H, OCOCH_3) 2.10 (s, 3H, OCOCH_3), 2.16 (s, 3H, OCOCH_3), 3.99 (ddd, $J_{5,6} = 2.5$, $J_{5,6'} = 5.0$, $J_{4,5} = 9.0$, 1H, H-5), 4.10 (d, $J_{1,1'} = 15.5$, 1H, H-1), 4.23 (dd, $J_{6,6'} = 12.5$, 1H, H-6), 4.27 (d, 1H, H-1′), 4.32 (dd, 1H, H-6′), 5.34 (t, $J_{3,4} = 10.0$, 1H, H-4), 5.42 (d, 1H, H-3). ^{13}C NMR ($CDCl_3$ at 77.0, 75.5 MHz) δ 20.4, 20.7 ($3 \times$ OCO*C*H$_3$), 62.1 (C-6), 69.4 (C-4), 72.9 (C-1), 76.5 (C-5), 76.8 (C-3), 169.1, 169.8, 170.5 ($3 \times$ O*C*OCH$_3$), 196.3 (C-2).

Anal. Calcd for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.87; H, 5.56.

3,6-Di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulose (8). To a solution of **7** (2.21 g, 7.67 mmol) in dry acetone (77 mL), anhydrous NaOAc (2.2 g, 26.8 mmol) was added and the reaction mixture was stirred for 3 h. The salts were filtered off and washed with acetone. The filtrate was concentrated and purified by column chromatography (30 g silica, eluted with hexane-EtOAc, 2:1) to give **8** as a syrup (1.56 g, 89%): $[\alpha]_D - 36.8$ (*c* 2.0, $CHCl_3$) [Lit.[7] $[\alpha]_D - 43.7$ (*c* 1.7, $CHCl_3$)]. ^{1}H and ^{13}C NMR are in accordance with literature.[7]

Anal. Calcd for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30. Found: C, 52.01; H, 5.18.

1,5-Anhydro-D-*glycero*-hex-3-en-2-ulose (9). Compound **8** (2.98 g, 13.1 mmol) was dissolved in aqueous 4 M HCl (130 mL) and kept for 24 h. The mixture was concentrated and co-concentrated with H_2O (2×60 mL) to a syrup, which was purified by chromatography (60 g silica, eluted with EtOAc, then $CHCl_3$-MeOH, 4:1) to give **9** together with isomeric and hydrated forms as an amorphous solid (1.84 g, 97%). ^{13}C NMR (the dihydrate **2**) (D_2O, MeOH at 49.5 ppm, 50.3 MHz) δ 37.4 (C-4), 64.2 (C-6), 70.9 (C-1), 76.4 (C-5), 92.9 (C-3), 93.9 (C-2). The product was used without further purification.

1,5-Anhydro-D-*glycero*-hex-1-en-3-ulose (1). Compound **9** (1.04 g, 7.2 mmol) was dissolved in dry pyridine (100 mL) and 4A molecular sieves (10.8 g) added. The mixture was heated at 120°C in an atmosphere of N_2 for 1 h and concentrated *in vacuo* to give a syrup, which was dissolved in H_2O (50 mL) and acidified with 1 M HCl until pH 4–5. The aqueous phase was extracted with EtOAc (5×100 mL) and the combined organic phase was dried ($MgSO_4$) and concentrated to a brown syrup. Upon addition of EtOAc-hexane, **1** crystallised (0.190 g, 18%): mp 90–95°C; $[\alpha]_D + 139.5$ (*c* 1.0, H_2O)

[Lit.[1] mp 98.5–99°C, $[\alpha]_D$ +155 (*c* 1.1, H_2O)]. The mother liquer was purified by chromatography (20 g silica, eluted with EtOAc, then $CHCl_3$-MeOH, 4:1) to afford **9** (0.57 g) and **1** (0.0494 g). Total yield of **1**: 23% (51% when subtracting recoved starting material). ^{1}H NMR (D_2O, MeOH at 3.34 ppm, 300 MHz) δ 2.53 (ddd, $J_{4,6}$ = 1.0, $J_{4,5}$ = 3.5, $J_{4,4'}$ = 17.5, 1H, H-4), 2.87 (ddd, $J_{4',6'}$ = 1.0, $J_{4',5}$ = 14.5, 1H, H-4′), 3.79 (ddd, $J_{5,6}$ = 5.5, $J_{6,6'}$ = 12.5, 1H, H-6), 3.88 (ddd, $J_{5,6'}$ = 3.0, 1H, H-6′), 4.57 (m, 1H, H-5), 7.53 (s, 1H, H-1). ^{13}C NMR (D_2O, MeOH at 49.5 ppm, 75.5 MHz) δ 37.7 (C-4), 63.7 (C-6), 81.0 (C-5), 136.1 (C-2), 152.3 (C-1), 192.9 (C-3).

Anal. Calcd for $C_{10}H_{12}O_6$: C, 50.00; H, 5.59. Found: C, 50.86; H, 5.98.

1,5-Anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose 2,4-dinitrophenylosazone (10a).[14,18] 2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (**5**)[15,16] (3.0 g, 9.1 mmol) was dissolved in dry MeOH (20 mL) and kept at 0°C. MeOH saturated with ammonia (10 mL) was added and the reaction kept at 0°C overnight. The mixture was concentrated in vacuo at ambient temperature and co-concentrated with dry MeOH (2 × 10 mL). Finally, the mixture was dissolved in dry EtOH (9 mL) and by addition of dry Et_2O (44 mL) an oily product was obtained (1.8 g). To a filtered solution of 2,4-dinitrophenylhydrazine (6.0 g, 30.3 mmol) in 2 M HCl (1.0 L) the oily product was added and the mixture was stirred overnight at ambient temperature. The precipitate was filtered off and washed with H_2O (2 × 20 mL) and dry EtOH (2 × 20 mL) affording the osazone **10a** (3.05 g, 67% yield from **5**, mp 239–242°C). Recrystallisation in EtOH-acetone afforded an analytical sample: mp 246°C. The sample was too coloured to obtain an optical rotation. [Lit.[14] mp 237°C, $[\alpha]_D$ +176 (pyridine)]. ^{1}H NMR (DMSO-d_6 at 2.50, 300 MHz) δ 2.74 (dd, $J_{4,5}$ = 10.5, $J_{4,4'}$ = 17.5, 1H, H-4), 3.00 (dd, $J_{4',5}$ = 4.0, 1H, H-4′), 3.56 (t, J = 4.5, 2H, H-6 and H-6′, *AB*X-system), 3.86 (dddd, J = 4.5, 4.5, 4.5, 10.5, 1H, H-5, AB*X*-system), 4.51 (d, $J_{1,1'}$ = 15.0, 1H, H-1), 4.64 (d, 1H, H-1′), 5.03 (t, $J_{OH,6}$ = $J_{OH,6'}$ = 4.5, 1H, -OH), 8.0–8.9 (6H, 2,4-dinitrophenyl), 11.07 (broad s, 1H, N*H*), 13.80 (broad s, 1H, N*H*). ^{13}C NMR (DMSO-d_6 at 39.5, 50.3 MHz) δ 28.8 (C-4), 63.3 (C-6), 70.2 (C-1), 75.1 (C-5), 116.8, 117.4, 122.5, 129.6, 130.0, 130.9, 131.3, 138.6, 138.9 (2,4-dinitrophenyl), 140.0 (C-3), 142.7, 144.6 (2,4-dinitrophenyl), 148.7 (C-2).

Anal. Calcd for $C_{18}H_{16}N_8O_{10}$: C, 42.86; H, 3.20; N, 22.22. Found: C, 43.11; H, 3.18; N, 22.02.

6-*O*-Acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose phenylosazone (12).[13] To a solution of 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (**5**)[15,16] (2.37 g, 7.16 mmol) in 50% AcOH (36 mL), $PhNHNH_2$ (2.9 mL, 29.4 mmol) was added. The reaction mixture was refluxed for a few minutes, until the product started precipitating, and then stirred at rt for 2 h. The mixture was poured onto ice (150 g) and the precipitated product **12** was filtered off (1.90 g, 73%, mp 176–178°C). Two recrystallisations from EtOH afforded an analytical sample: mp 203–204°C; $[\alpha]_D$ −120.1 (*c* 1.0, $CHCl_3$) [Lit.[13] mp 205°C, $[\alpha]_D$ −98.6 (pyridine)]. ^{1}H NMR ($CDCl_3$ at 7.27, 300 MHz) δ 2.16 (s, 3H, OCOC*H*$_3$), 2.44 (dd, $J_{4,5}$ = 11.0, $J_{4,4'}$ = 16.5, 1H, H-4), 2.63 (dd, $J_{4',5}$ = 4.5, 1H, H-4′), 3.97 (dddd, $J_{5,6'}$ = 4.0, $J_{5,6}$ = 6.0, 1H, H-5), 4.22 (dd, $J_{6,6'}$ = 12.0, 1H, H-6), 4.32 (dd, 1H, H-6′), 4.46 (d, $J_{1,1'}$ = 15.0, 1H, H-1), 4.70 (d, 1H, H-1′), 6.87–7.43 (10H, phenyl), 7.45 (broad s, 1H, N*H*), 12.65 (broad s, 1H, N*H*). ^{13}C NMR ($CDCl_3$ at 77.0, 75.5 MHz) δ 20.9 (OCO*C*H$_3$), 27.2 (C-4), 66.0 (C-6), 71.1 (C-1),

72.4 (C-5), 113.4, 120.8, 121.7 (phenyl), 127.9 (C-3), 129.3, 129.6 (phenyl), 137.9 (C-2), 143.6, 144.4 (phenyl), 170.9 ($OCOCH_3$).

Anal. Calcd for $C_{20}H_{22}N_4O_3$: C, 65.56; H, 6.05; N, 15.29. Found: C, 65.30; H, 6.24; N, 15.06.

1,5-Anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulose phenylosazone (10b).[13] To the phenylosazone **12** (1.03 g, 2.81 mmol) was added 0.5 M NaOMe/MeOH (30 mL) at 0°C and the mixture was stirred for 1/2 h at 0°C. The reaction mixture was acidified (pH < 6) with AcOH and concentrated to a syrup, from which **10b** crystallised upon addition of 10% AcOH (150 mL). The precipitate was washed with H_2O (2×20 mL) to afford **10b** (0.74 g, 82%, mp 162–164°C). Two recrystallisations from EtOH-H_2O afforded an analytical sample: mp 180–182°C; $[\alpha]_D$ −141.6 (*c* 1.0, pyridine) [Lit.[13] mp 183°C, $[\alpha]_D$-159.8 (pyridine)]. ^{1}H NMR (DMSO-d_6 at 2.50, 300 MHz) δ 2.42 (dd, $J_{4,5} = 11.0$, $J_{4,4'} = 17.5$, 1H, H-4), 2.77 (dd, $J_{4',5} = 4.0$, 1H, H-4′), 3.55 (t, J = 5.5, 2H, H-6 and H-6′, *AB*X-system), 3.75 (dddd, J = 4.5, 4.5, 4.5, 11.0, 1H, H-5, AB*X*-system), 4.36 (d, $J_{1,1'} = 14.5$, 1H, H-1), 4.47 (d, 1H, H-1′), 4.93 (t, $J_{OH,6} = J_{OH,6'} = 5.5$, 1H, -OH), 6.79–7.41 (10H, phenyl), 9.67 (broad s, 1H, N*H*), 12.75 (broad s, 1H, N*H*). ^{13}C NMR (DMSO-d_6 at 39.5, 50.3 MHz) δ 28.4 (C-4), 63.9 (C-6), 70.0 (C-1), 75.2 (C-5), 112.6, 113.0, 120.2, 120.4, 129.5 (phenyl), 130.4 (C-3), 139.3 (C-2), 144.4, 144.6 (phenyl).

Anal. Calcd for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.40; H, 6.16; N, 17.39.

Assay of Antioxidant Activity of Ascopyrone P (1). Ascopyrone P was evaluated for possible antioxidative activity by measuring its inhibition on the formation of oxidation products from the polyunsaturated fatty acid, linolenic acid. The oxidation products formed, lipid hydroperoxide, malonadehyde (MDA) and 4-hydroxyalkenals (4-HNE), were measured using the assay kit of Bioxytech® LPO-560 and Bioxytech® LPO-586 from Oxis International, Inc. (Portland, USA). The lipid hydroperoxide assay is based on its oxidation of Fe^{2+} to Fe^{3+}; the formed Fe^{3+} gives colour with xylenol orange having a maximum absorbance at 560 nm. Linolenic acid (20 μL) was dissolved in oxygenated water (50 mL) containing Tween 40 (0.2 mL) (Solution 1). To solution 1 (0.96 mL) was added 0.1 M EDTA (20 μL) and water (20 μL) (control) or ascopyrone P (20 μL) (experimental) with a final concentration of 160 ppm. The reaction mixture was incubated at 24°C in the dark. At the 6th day, samples (0.1 mL) were taken and mixed with working solution from the assay kit containing Fe^{2+} xylenol orange (0.9 mL). Absorbance at 560 nm was measured after incubation at 24°C in the dark for 60 min against reagent blank, which had the same composition as the control and experimental groups, but not incubated. With no antioxidant added the absorbance was 0.748. In the presence of 160 ppm ascopyrone P the absorbance was 0.397. The assay for MDA and 4-HNE is based on their reaction in acid with the chromogenic reagent 1-methyl-2-phenylindole at 45°C to form a stable chromophore that has maximum absorbance at 586 nm. At the 6th day samples (40 μL) were taken and after mixing with 0.5 M BHT (5 μL), freshly diluted *N*-methyl-2-phenylindole (7.7 mM, 130 μL) and methanesulfonic acid (15.4 M, 30 μL) were added and mixed. The reaction mixture was incubated at 45°C for 45 min and cooled to ambient temperature and the absorbance at 590 nm was measured using a micro plate reader within 2 hours.

Without antioxidant the absorbance was 0.280. In the presence of 160 ppm ascopyrone P the absorbance was 0.116.

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