In memory of Friedrich Cramer

(--)-Daucic acid, a C7-sugar dicarboxylic acid found in carrots (*Daucus carota*), sugar beet, wheat, sunflowers, and tobacco, was first isolated from mature carrots in 1971 [1] and later assigned the structure of a 2,6-anhydro-3-deoxy-β-hept-2-enaric acid and d-xylo configuration with respect to the three chiral centers (Scheme 1, 1). [2] The assignment of a dihydro-

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**Scheme 1.** Reactions of (--)-daucic acid, isolated from *Daucus carota*, from which the structure of a 2,6-anhydro-2-deoxyhept-2-enaric acid was derived; the d-xylo configuration 1 was determined on the basis of 1H NMR spectroscopic data (60 MHz). [2]

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The assignment of the configuration at C4 and C5 was less conclusive (Scheme 1), as it was inferred from low-resolution (60 MHz) $^1$H NMR spectroscopic data for dimethyl daucate (2) and its di-$O$-acetyl derivative, primarily from the coupling constants for 4-H and 5-H and comparison of these with analogous NMR spectroscopic data for certain 4,5-unsaturated hexuronic acids,[4] which on close inspection prove unreliable.[5] Moreover, the half-chair forms of sugar-derived dihydropyrans usually exist in complex conformational equilibria, which are virtually impossible to predict, thus resulting in coupling patterns that have little bearing on the actual configuration. Therefore, the verification of the stereochemical coupling patterns that have little bearing on the actual stereochemistry at C4 and C5 of (−)-daucic acid was deemed imperative, particularly as reflections on its biosynthetic origin—its formation from 3-deoxy-$d$-arabinose-heptulosonate 7-phosphate (DAHP), an early intermediate of the shikimic acid pathway,[6] is a likely possibility—would lead one to expect the $d$-arabino configuration.

These considerations, together with the notion that daucic acid is a possible biosynthetic precursor of chelidonic acid (4), a leaf-closing factor in Cassia mimosoides,[7] prompted us to devise a stereocontrolled synthesis, which should be practical enough to furnish sufficient amounts for biological studies. Accordingly, herein we report expedient syntheses of the daucic acids with the $d$-xylo, $d$-ribo, $l$-arabino, and $d$-lyxo configuration. Our concept was for developing stereochemically unambiguous access to the $d$-xylo-heptenonic acid 1 and the three alternate configurations of this acid was based upon the anomic one-carbon-atom homologation of suitable $d$-hexoses ($d$-galactose or $d$-mannose), subsequent oxidation at both termini to the corresponding pyranoid C7 dicarboxylic acids, and controlled $\beta$ elimination into the pyranoid ring through the judicious choice of leaving groups.

The synthesis of the dimethyl $d$-xylo-dicarboxylate 2 started from tri-$O$-acetyl-2-acetoxy-$d$-galactal (7), readily accessible from $d$-galactose in a three-step, one-pot procedure[8] involving acetylation, treatment with HBr/HOAc, and dimethylanime-promoted elimination of HBr (Scheme 2). The acetone-initiated photoaddition of formamide to 7, albeit complex,[9] is a selective to give the heptonamide 8 as the major product (54 %), which can be converted into the methyl heptonate 9 by methanolation under acidic conditions. Oxidation of the primary hydroxy group was effected smoothly with oxygen in the presence of the Adams catalyst to afford, after esterification with methanolic HCl, the dimethyl heptonate 10. Although the axial orientation of the S-OH group in 10 would suggest preferential 5,6-elimination from the corresponding tri-$O$-acetyl (11) or tri-$O$-benzoyl derivative, their exposure to a variety of suitable conditions (e.g. NaOAc/Ac$_2$O, 70°C or Al$_2$O$_3$/lutidine, 40°C) led to multicomponent mixtures. Thus, a better leaving group had to be introduced at C5. Low-temperature benzyolation of the equatorial hydroxy groups in 10 and subsequent treatment with methanesulfonyl chloride provided 12. Now 5,6-elimination could be effected cleanly, either from 12 by briefly heating in NaOAc/Ac$_2$O to afford the dibenzoate 15 (59 %), or from the debenzyolated product 13 through exposure to NaOMe/Methanol to deliver dimethyl $d$-xylo-heptenonate 2 (77 %) directly. Gratifyingly, all products in this reaction sequence were obtained in readily characterizable, crystalline form; only the di-$O$-acyl and di-$O$-benzoyl derivatives 14 and 15 have so far resisted crystallization.

The melting point for the product 2 of this reaction sequence was close to that of the Daucus carota derived compound. However, its optical rotation, although similar in size, was opposite in sign (Table 1). The notion that the natural product might therefore have the enantiomeric (i.e. $L$-xylo) configuration was invalidated by the distinct differences in the $^1$H NMR spectroscopic data of synthetic 2 and 14, compared to those reported for the respective products of natural origin. The chemical shifts observed for 4-H, 5-H, and 6-H vary by $\delta = 0.3–0.6$ ppm in each case, and the coupling constants $J_{4,5}$ and $J_{3,4}$ have significantly different values (Table 1). Thus, a $d$-xylo or $l$-xylo configuration for natural (−)-daucic acid can be excluded unequivocally.

Of the remaining possible configurations—$d$-ribo, $d$-lyxo, and $d$-arabino—the synthesis of the $d$-ribo analogue 22, the C5 epimer of 2, was addressed next. We took advantage of the ready accessibility of heptononitrile 16 from $d$-mannose through a two-step, one-pot reaction comprising acetylation and anomeric cyanation (Scheme 3).[10] Acid hydrolysis followed by esterification with methanol then provided the mannosyl-C-carboxylate 17. The PtO$_2$ oxidation of the primary hydroxy group in 17 proved unusually capricious,
and the conversion of this compound into the desired dicarboxylate was instead effected by a TEMPO-catalyzed oxidation with sodium hypochlorite,[11] to provide, upon esterification with methanolic HCl, the dimethyl heptenate 18 in high yield. Subsequent protection of the hydroxy groups at C3 and C4 through acetonide formation (19), followed by mesylation (20), set the stage for the 5,6-elimination of methanesulfonic acid, which was effected simply by briefly exposing 20 to Al2O3/lutidine at 40 °C (21). Finally, removal of the isopropylidene group in 21 with aqueous trifluoroacetic acid smoothly delivered the desired dimethyl d-ribo-heptenate 22, which was acetylated to the di-O-acetyl derivative 23. Both 22 and 23 were isolated as syrups with high positive optical-rotation values. Substantially smaller negative values were observed for the Daucus carota derived product. As distinct differences were also observed in the respective 1H NMR spectroscopic data (see Table 1), the d-ribo and l-arabino stereochemistry for natural (−)-daucic acid can also be excluded.

To finally unravel the stereochemistry of the natural product—arabino or lyxo configurations were still possible—we investigated a convergent synthetic pathway from β-d-galactosyl cyanide 24,[12] under the premise that Δ5,6 (or Δ5,7) unsaturation could be introduced selectively through an elimination reaction if the 3-OH (or, alternatively, the 5-OH) group in the C7-dicarboxylate 26 was converted into a displacable leaving group (Scheme 4). Indeed, this key intermediate was prepared in a straightforward manner by

### Table 1: Relevant physicochemical data for dimethyl 2,6-anhydro-3-deoxyhept-2-enarates of d-xylo, d-ribo, l-arabino, and d-lyxo configuration and their diacetates, as compared with those reported for the analogous Daucus carota derived daucic acid derivatives.[2]

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.p. [°C]</th>
<th>[α]D[20][a]</th>
<th>4-H</th>
<th>5-H</th>
<th>6-H</th>
<th>1H NMR [ppm, Hz]</th>
<th>J1,4</th>
<th>J1,5</th>
<th>J4,5</th>
<th>J5,6</th>
<th>solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 R = H</td>
<td>133–135</td>
<td>+106</td>
<td>4.19</td>
<td>4.60</td>
<td>4.8</td>
<td>1.3</td>
<td>1.5</td>
<td>1.8</td>
<td>CDCl3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 R = Ac</td>
<td>syrup</td>
<td>+146</td>
<td>5.13</td>
<td>5.38</td>
<td>4.61</td>
<td>5.3</td>
<td>1.5</td>
<td>2.2</td>
<td>1.4</td>
<td>CDCl3</td>
<td></td>
</tr>
<tr>
<td>22 R = H</td>
<td>syrup</td>
<td>+150.4</td>
<td>4.32</td>
<td>4.12</td>
<td>4.63</td>
<td>4.4</td>
<td>−</td>
<td>4.3</td>
<td>7.6</td>
<td>CDCl3</td>
<td></td>
</tr>
<tr>
<td>23 R = Ac</td>
<td>syrup</td>
<td>+171.1</td>
<td>5.52</td>
<td>4.84</td>
<td>3.7</td>
<td>−</td>
<td>?</td>
<td>6.8</td>
<td>CDCl3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 R = H</td>
<td>syrup</td>
<td>+30.0</td>
<td>4.25</td>
<td>4.19</td>
<td>4.73</td>
<td>4.3</td>
<td>0.9</td>
<td>2.2</td>
<td>5.4</td>
<td>CDCl3</td>
<td></td>
</tr>
<tr>
<td>30 R = Ac</td>
<td>104–106</td>
<td>+72.1</td>
<td>5.14</td>
<td>5.44</td>
<td>5.06</td>
<td>5.4</td>
<td>1.5</td>
<td>2.1</td>
<td>2.2</td>
<td>CDCl3</td>
<td></td>
</tr>
<tr>
<td>35 R = H</td>
<td>128–129</td>
<td>−98.3</td>
<td>4.50</td>
<td>4.30</td>
<td>4.67</td>
<td>3.3</td>
<td>1.1</td>
<td>4.3</td>
<td>2.3</td>
<td>CDCl3</td>
<td></td>
</tr>
<tr>
<td>36 R = Ac</td>
<td>syrup</td>
<td>−54.1</td>
<td>5.74</td>
<td>4.84</td>
<td>3.2</td>
<td>2.2</td>
<td>1.7</td>
<td>?</td>
<td>1.4</td>
<td>CDCl3</td>
<td></td>
</tr>
</tbody>
</table>

[a] Optical-rotation values for the free diols in acetone, for the diacetates in CHCl3. [b] [α]D value at 24.5 °C.

Scheme 3. Synthesis of the d-ribo-heptenate 22 from d-mannose:

a) pyridine/ACO (1.3:1), 0 °C, 2 days,[10a] then Me3SiCN, BF3·Et2O, CH3NO2, 50 °C, 24 h; b) TEMPO/NaOCl, H2O/CH2Cl2, 0 °C, 20 h, then saturated methanol HCl; c) aqueous HCl (25%), 50 °C, 24 h, then saturated methanol HCl; d) Me2CO/H2SO4, RT, 2 h, 83%; e) pyridine/Ac2O (2:1), RT, 5 h, 91%. TEMPO = 2,2,6,6-tetramethylpiperidinyl-1-oxyl, TFA = trifluoroacetic acid.
The elaboration of the d-fuco-configuration to carry-derived (−)-daucic acid.

The synthetic (−)-dimethyl daucate (35) could be saponified readily by exposure to aqueous trifluoroacetic acid (4:1, 2 days, 25 °C), to provide the free acid in crystalline form (m.p. 87–88 °C, [α]D = −85 in MeOH, 80 °C). More vigorous acid conditions led to the pyran—furan rearrangement observed previously for the natural product (Scheme 1)[2]

Heating 35 in methanolic HCl afforded dimethyl osbeckate (5) (60%), whereas heating 35 in water in the presence of a strongly acidic ion-exchange resin gave the free osbeckic acid (6; 81%).

A similar sequence of reactions led from the isopropyli-
dene-protected dimethyl heptarate 31 to the corresponding t-l-lyxo compounds 35 and 36. Mesylation (−33) and Al2O3/ lutidine-induced elimination gave 34, which was then exposed to aqueous trifluoroacetic acid to remove the isopropyli-
dene group.

Unlike the l-arabino-heptenarates 29 and 30, whose 1H NMR spectroscopic data differed markedly from those of the Daucus carota derived compounds[2] the d-fuco analogues 35 and 36 showed almost perfect agreement, not only in the sign and magnitude of their optical-rotation values, but most notably in the chemical shifts and coupling patterns in their 1H NMR spectra (Table 1). The slight deviations observed in the coupling constants undoubtedly result from the different field strengths (60[2] versus 300 MHz) and, conceivably, from temperature differences. The temperature affects the equilibrium between the respective half-chair forms (1H, e = 1H) and hence the NMR coupling patterns observed. This evidence is cogent enough to allow the unambiguous assignment of the d-fuco-configuration to carry-derived (−)-daucic acid.

Not only is the biosynthetic origin of (−)-daucic acid intriguing—sedoheptulose 7-phosphate (37) and 3-deoxy-d-arabinono-heptulosonate 7-phosphate (DAHP, 38), both established intermediates of the pentose phosphate and shikimic acid pathways, respectively, are potential precursors—but also its close structural relationship with chelidonic acid (4).

The elaboration of the y-pyrene system would merely require oxidation of daucic acid at C4 and 5,6-elimination of water.

Studies on the biosynthesis of chelidonic acid with 14C-labeled sugars have uniformly shown that d-glucose, and to an even greater extent d-ribose, are incorporated well, [13,14] whereas 37 is not.[13] Although the assumption that DAHP (38) is therefore the likely precursor[14] persisted for 30 years, it was convincingly disproved recently by quantitative carbon-flux analyses of 14C-labeled sugars, which suggested a biosynthetic assembly of chelidonic acid from one molecule each of pentose phosphate and phosphoenolpyruvate (PEP;
Such a biosynthetic process is well-established for Gram-negative bacteria, in which PEP and α-arabinose 5-phosphate (α-Ara 5-P), in turn generated from α-ribose 5-phosphate by isomerization, undergo an aldol-type condensation to form 3-deoxy-D-manno-octulosonate 8-phosphate (KDO 8-P, Scheme 5).[18] As the KDO 8-P synthase catalyzes aldol addition proceeds with exclusive Si attack by PEP to the Re face of the sugar carbonyl group, the 4R isomer is formed stereospecifically, so that the product 39 has the D-manno configuration.[18] The existence of such a KDO 8-P based mechanism in plants has not yet been proved. However, the fact that DAH 7-P synthase, a key enzyme of the shikimic acid pathway,[4] and abundant in higher plants, catalyzes the aldol addition of PEP not only to its natural substrate d-erythrose 4-phosphate, but to d-ribose 5-phosphate (d-Rib 5-P) and d-Ara 5-P as well,[19] may be regarded tentatively as evidence that an octulosonate 8-phosphate based pathway is also operative in plants.

Based on the newly established d-lyxo stereochemistry of (−)-daucic acid (37 and 38 as precursors would lead to the D-arabinino configuration), on the conjecture that the biosynthetic elaboration of daucic acid is closely related to that of chelidonic (4) and meconic acid (44), and on available evidence[10] about the biosynthesis of 4, a biosynthetic pathway readily unfolds as depicted in Scheme 5: d-Ara 5-P and PEP undergo an aldol-type condensation to KDO 8-P (39), in which the stereochemistry of the pyranoid ring correlates with the d-lyxo configuration of daucic acid. If d-Rib 5-P was involved in the aldol addition step, the C₆-sugar phosphate would have the d-altro configuration, thus requiring an (unnecessary) epimerization at a later stage.

Unlike in Gram-negative bacteria, in which the C₆ chain of KDO 8-P is incorporated into the lipopolysaccharides of cell walls,[10] in this case the terminal carbon is removed by dephosphorylation and oxidative decarboxylation. Although only loss of water is formally required from the resulting intermediate 40 to give daucic acid, a direct 3,2-elimination is unlikely—“cells obey the laws of chemistry”[20]—as the hydrogen atom involved (3-H) is not activated. As activation is usually provided by a vicinal carbonyl group—the conversion of d-glucose into kojic acid by Aspergillus oryzae has been rationalized on this basis[21]—the oxidation of 40 at C4 appears plausible as the next step. Dehydration of the resulting ketodicarboxylic acid 41 is thought to proceed spontaneously to give the dihydropyranone 42, a furcation point towards these C₇ dicarboxylic acids. Daucic acid is formed from 42 by reduction (42 → 43), chelidonic acid by elimination of water (42 → 4), and meconic acid, plentiful in Papaveraceae, by oxidation (42 → 44). The furanoid osbeckic acid is likely to be formed from daucic acid through ring contraction and double dehydration (43 → 6).

The alluring consistency of this proposed biosynthetic pathway, particularly the configurational identity of KDO 8-P and daucic acid in their pyranoid rings, clearly calls for the systematic investigation of higher plants for the occurrence of a C₇-sugar-phosphate pathway with the possible intermediates 40–42, in particular in those species in which these C₇ dicarboxylic acids have been detected: daucic acid in carrots, sugar beets, wheat, sunflowers, and tobacco, osbeckic acid in Osbeckia chinensis L., meconic acid in Papaveraceae, and chelidonic acid in a plethora of plant families.

Keywords: carbohydrates · chelidonic acid · configuration determination · daucic acid · osbeckic acid

References
materials III–V and VII–XI are identical, yet lead to different products, namely, to a β anomer VI and an α anomer X; moreover the coupling constants for the β (VI) and α compound (X) seem to have been interchanged, as only the latter can adopt a half-chair conformation with a H2-C-C-H3 torsion angle of \( \approx 130^\circ \), and hence result in a coupling constant \( J_{2,3} = 7.5 \text{ Hz} \). Consequently, these data are not useful for comparative purposes.


