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## 109. 3-*C*-Hydroxymethyl-D-riburonic Acid Synthesis of the Branched-Chain Uronic Acid and Comparison with a Carbohydrate Component of a Naturally Occurring Bilirubin Conjugate

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(22. III. 74)

*Summary.* A novel carbohydrate has previously been isolated from human bile as a complex glycoside of bilirubin [1]. This compound has been tentatively identified as 3-*C*-hydroxymethyl-D-riburonic acid. To test this structural assignment the proposed branched-chain uronic acid was synthesized. Gas chromatographic and mass spectrometric comparison of the natural and synthetic

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materials indicated that the proposed structure, 3-*C*-hydroxymethyl-D-riburonic acid, did not apply to the natural product.

Bilirubin, the major bile pigment in humans, is excreted by the liver in the form of glycosides. These are referred to in the medical literature as bilirubin conjugates. The carbohydrate portions of these natural bilirubin derivatives consist of various disaccharides. One of these is composed of glucuronic acid and what appears to be a branched-chain uronic acid. The latter has been tentatively identified as 3-*C*-hydroxymethyl-*D*-riburonic acid. We synthesized this compound two years ago with the aim of identifying the naturally occurring substance. *Paulsen & Stenzel* [2] have since prepared this compound by an alternative route. Here we present our own synthetic procedure and report on the comparison of the natural and synthetic materials.

1,2 : 5,6-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-allofuranose (**1**) was prepared from 1,2 : 5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose [3] by reaction with excess nitromethane in the presence of 0.1 mol equivalent of potassium *t*-butoxide as described by *Albrecht & Moffatt* [4]. These authors have shown that under the conditions adopted the nitromethyl carbanion adds stereospecifically to the carbonyl function to yield the *allo*-epimer exclusively. Permanganate oxidation of the nitro group by a modified *Nef* reaction [5] afforded the aldehyde **2**, which was not isolated but was directly converted to the crystalline hydroxymethyl derivative **3** by borohydride. The primary hydroxyl group was then protected by acetylation to render it resistant to subsequent periodate cleavage (see below). The resulting acetoxymethyl compound **4** was subjected to mild acid treatment to selectively remove the 5,6-*O*-isopropylidene group. The crystalline 5,6-diol **5** so obtained was cleaved by periodate in an attempt to prepare the aldehyde. After passage through a mixed-bed ion-exchange resin the reaction mixture was found by thin-layer chromatography ($SiO_2$, ether) to consist of a mixture of two products. The major product (Rf 0.3, m.p. 112–115°, $[\alpha]_D^{22} + 72°$ in $CHCl_3$) was shown by IR. (no carbonyl absorption), NMR. (two OH, no OAc), MS. (*m/e* 203, consistent with $M^+ - \dot{C}H_3$) and elemental analysis to be the hemiacetal **6**. *Paulsen & Stenzel* [2], having prepared this compound by an alternative route, report m.p. 115–116° and $[\alpha]_D^{20} - 28°$. This discrepancy might be explained by invoking preferential formation of the two alternative epimers at C(5) depending on the reaction conditions employed. However, since both epimers may be expected to give the same lactone **7** upon further oxidation, the stereochemistry at C(5) is not a relevant feature of this intermediate in the synthesis of 3-*C*-hydroxymethyl-D-riburonic acid. Besides the hemiacetal **6** periodate cleavage also afforded a minor product (Rf 0.5), to which the structure of the expected aldehyde was tentatively assigned mainly on the basis of its property of being converted to the more polar compound by repeated passages through the ion-exchanger. The mechanism by which this conversion occurred seemed to be a basic resin catalysed de-acetylation followed by an acid resin catalysed cyclisation to the hemiacetal. Following the initial observation this behaviour was exploited subsequently on a preparative scale. The hemiacetal **6** so obtained was subjected to a modified *Pfitzner-Moffatt* oxidation [3] [6] to yield the lactone **7**, m.p. 130–133°, $[\alpha]_D^{22} + 55°$ in $CHCl_3$ (*Paulsen & Stenzel* [2] report m.p. 134°, $[\alpha]_D^{20} + 57°$). Removal of the isopropylidene group by dilute acid gave a mixture of 3-*C*-hydroxymethyl-D-riburonic acid (**10**) together with its lactones

**8** and **9**. This mixture was trimethylsilylated and analysed by a combination of GLC. and MS. The results are presented in Fig. 1 and Table 1. Their comparison with similar data obtained by *Stenzel* [2] seems hardly possible since in the cited work GC. resolution was incomplete thus preventing the collection of mass spectra from individual components of the mixture.

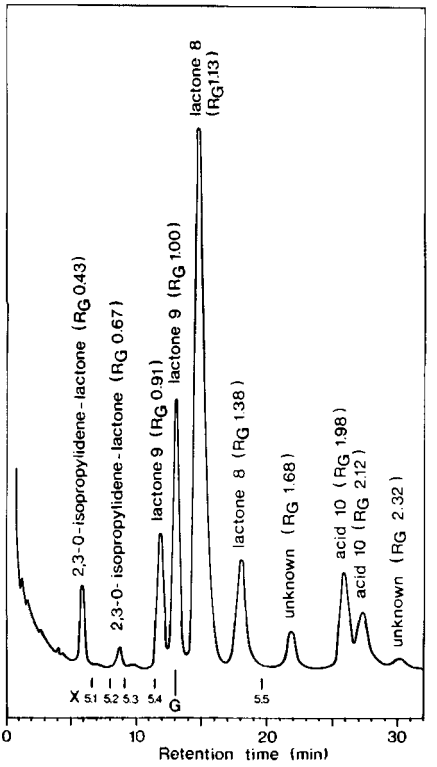



Fig. 1. *Gas-liquid chromatogram obtained after trimethylsilylation of the acid hydrolysate of* **7**. Peaks were tentatively identified by mass spectrometry (see Table 1 and text). Retention times ($R_G$) relative to β-glucuronolactone are given for each peak. Vertical marks indicate the retention times of β-glucuronolactone and of the natural compounds ($X_{5.1}$ to $X_{5.5}$) observed previously in an analysis of bile constituents [1]. The unavailability of these compounds necessitated their plotting from calculations based on their relative retention times ($R_G$) determined earlier.

The two earliest peaks on the chromatogram (retention times relative to β-glucuronolactone, $R_G$ 0.43 and $R_G$ 0.67) were identified as 2,3-*O*-isopropylidene isomers of the lactone **7** generated by acid catalysed rearrangement of the isopropylidene group from the 1,2- to the 2,3-position. This interpretation was derived from the mass spectra of the two peaks, which were consistent with monoacetonide structures (for $R_G$ 0.43, $M^+$ at *m/e* 288, $M^+ - \dot{C}H_3$ at *m/e* 273; for $R_G$ 0.67, $M^+ - \dot{C}H_3$ at *m/e* 273) but differed from the spectrum of trimethylsilylated lactone **7**. Furthermore, the latter ($R_G$ 0.57) did not coincide chromatographically with either of the two peaks.

Next on the chromatogram came a cluster of four peaks with $R_G$ 0.91, 1.00, 1.13 and 1.38. All gave mass spectra consistent with the expected lactones **8** and **9**. The fact that compound **7** upon hydrolysis gave rise to four lactones was further proof of the correct synthesis since 3-*C*-hydroxymethyl-riburonic acid is the only conceivable six-carbon uronic acid with the potential to form four lactones [1]. Further, mass

Table 1. *Mass spectra of trimethylsilyl derivatives of 3-C-hydroxymethyl-D-riburonic acid and its lactones.* Mass spectra were recorded from gas chromatographic peaks having retention times relative to β-glucuronolactone, $R_G$ 0.91, $R_G$ 1.38 and $R_G$ 1.98 (compare Fig. 1).

| m/e | Relative intensity of signal (% of base peak) | | | m/e | Relative intensity of signal (% of base peak) | | |
|---|---|---|---|---|---|---|---|
| | Peak $R_G$ 0.91 | Peak $R_G$ 1.38 | Peak $R_G$ 1.98 | | Peak $R_G$ 0.91 | Peak $R_G$ 1.38 | Peak $R_G$ 1.98 |
| 539 | – | – | 0.3 ($M^+ - \dot{C}H_3$) | 204 | 2.4 | 2.2 | 3.4 |
| 449 | – | – | 0.8 | 192 | 1.7 | 1.9 | 9.0 |
| 393 | – | – | 0.8 | 191 | 14.0 | 10.3 | 50.3 |
| 392 | 0.9 ($M^+$) | 0.3 ($M^+$) | – | 189 | 1.9 | 1.7 | 3.8 |
| 378 | 1.3 | 0.4 | – | 185 | 1.3 | 2.5 | – |
| 377 | 4.2 | 1.1 | – | 184 | 2.0 | 1.0 | – |
| 359 | – | – | 1.9 | 177 | 0.8 | 1.8 | – |
| 349 | 1.2 | – | – | 173 | 1.2 | 1.6 | – |
| 347 | – | – | 3.0 | 171 | 0.9 | 0.6 | – |
| 331 | – | – | 9.2 | 169 | 1.4 | 4.2 | 0.8 |
| 319 | 1.2 | 2.7 | 6.8 | 157 | 3.7 | 4.2 | 3.2 |
| 305 | – | – | 3.2 | 156 | 1.9 | 3.5 | 0.6 |
| 292 | – | – | 2.4 | 149 | 6.1 | 7.1 | 4.0 |
| 287 | 2.2 | 1.1 | – | 148 | 5.4 | 7.7 | 4.7 |
| 285 | 3.1 | – | 1.9 | 147 | 32.8 | 44.8 | 29.0 |
| 274 | 1.4 | 1.1 | – | 143 | 2.8 | 1.8 | 2.1 |
| 261 | 0.6 | 3.0 | – | 141 | 0.6 | 3.8 | 0.6 |
| 260 | 0.8 | 6.5 | – | 140 | 1.4 | 1.6 | – |
| 259 | 3.2 | 29.3 | – | 133 | 9.0 | 8.4 | 5.1 |
| 257 | – | – | 1.8 | 131 | 2.0 | 3.6 | 2.3 |
| 248 | 3.5 | 0.8 | – | 130 | 17.8 | 1.1 | 1.2 |
| 247 | 8.3 | 1.1 | – | 129 | 11.5 | 7.2 | 7.6 |
| 246 | 37.0 | 2.5 | – | 117 | 3.4 | 2.4 | 2.3 |
| 245 | 5.1 | 11.2 | 2.8 | 115 | 1.6 | 0.9 | – |
| 244 | – | 2.4 | – | 113 | 3.4 | 1.4 | – |
| 243 | – | 2.2 | – | 103 | 7.4 | 5.2 | 3.7 |
| 232 | 3.2 | 1.1 | – | 102 | 14.3 | 0.7 | 1.0 |
| 231 | 14.0 | 4.0 | – | 101 | 2.1 | 2.0 | 1.1 |
| 230 | 4.5 | 7.2 | – | 99 | 2.1 | 1.5 | 0.6 |
| 229 | – | 3.8 | 2.6 | 75 | 21.1 | 16.8 | 13.6 |
| 218 | 4.1 | 1.8 m* 201 | 3.4 | 74 | 9.9 | 9.0 | 8.3 |
| 217 | 18.6 | 9.6 | 19.5 | 73 | 100.0 | 100.0 | 100.0 |
| 216 | – | 5.9 | 2.6 | | | | |
| 215 | 2.5 | 25.4 | 12.4 | | | | |

spectra of the four lactones revealed that they occurred in two groups as required by structures **8** and **9**. This was evident from the observation that the mass spectra of the peaks with $R_G$ 0.91 and $R_G$ 1.00 were almost identical, and the same applied to the pair of peaks with $R_G$ 1.13 and $R_G$ 1.38.

It remained to be determined which pair of lactones corresponded to compounds **8** and **9**, respectively. It has previously been proposed that the fragment of mass 245 could be used to discriminate between the two lactone forms. This was deduced from theoretical arguments suggesting that this fragment could arise from lactone **9** only, not however from lactone **8** [1]. Contrary to this, this fragment was present in

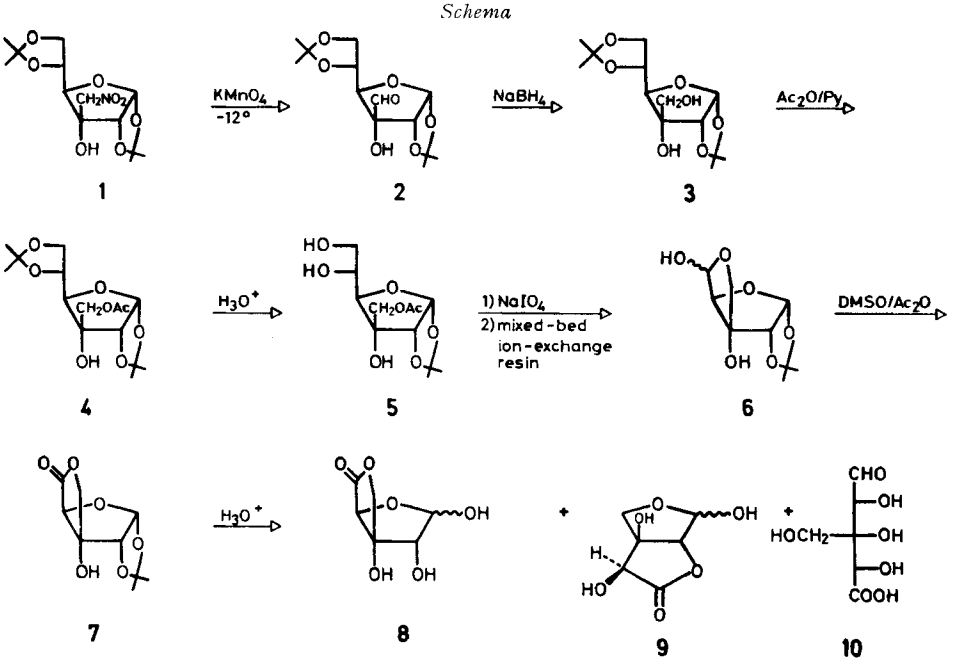
both groups of lactones (see Table 1 for the mass spectra of peaks with $R_G$ 0.91 and $R_G$ 1.38) and thus was of no value in this context. However, on the basis of high resolution mass spectrometry, *Stenzel* [2] has proposed that *m/e* 246 might serve as a key fragment to solve this issue. This fragment probably arises from lactone **9** by elimination of $\dot{C}H_3$, $CO_2$ and $\begin{matrix}\dot{C}H_2\\ CH_3\end{matrix}\!\!>\!Si\text{-}O\text{-}\dot{C}H_2$. Since the fragment of mass 246 was very prominent with the peaks having $R_G$ 0.91 and $R_G$ 1.00, but was of low intensity with the peaks having $R_G$ 1.13 and $R_G$ 1.38 (Table 1) it seemed probable that the former pair represented lactone **9** whereas the latter corresponded to lactone **8**.

The GLC. exhibited another four peaks, two of which could not be identified (peaks with $R_G$ 1.68 and $R_G$ 2.32). On the other hand, the peaks having $R_G$ 1.98 and $R_G$ 2.12 were revealed to be two of the expected four acids (Table 1), and the close similarity of the two spectra indicated that both peaks belonged to the same structural pair. However, no attempt was made to assign this pair to either of the two isomers corresponding to the lactones **8** and **9**, respectively.

Having thus identified the relevant peaks of the chromatogram it became possible to compare the synthetic material with the natural compound previously isolated as part of a bilirubin glycoside. The close duplication of previous experimental conditions allowed us to obtain meaningful analyses despite the fact that the natural compound was no longer available for direct comparison. Further, some obvious differences between the synthetic and natural compounds facilitated a decision and led us to rule out a possible identity.

For one, the GC. behaviour of the synthetic and natural compounds were sufficiently different to warrant such an interpretation (Fig. 1). Second, non-identity

*Schema*



was indicated by some mass spectrometric fragments (Table 1 and Ref. [1]), notably *m/e* 259 (very prominent in the synthetic lactone **8** (29%) but absent in the natural compounds $X_{5.1}$, $X_{5.2}$ and $X_{5.3}$), *m/e* 246 (very prominent in the synthetic lactone **9** (37%) but absent in the natural compounds $X_{5.1}$, $X_{5.2}$ and $X_{5.3}$), m/*e* 243 (absent or of low intensity (2%) in the synthetic lactones but very prominent in the natural compounds $X_{5.1}$ (37%) and $X_{5.2}$ (22%)), *m/e* 217 (prominent in the synthetic lactone **9** (18%) but of low intensity in the natural compounds $X_{5.1}$, $X_{5.2}$ and $X_{5.3}$ (1–4%)), *m/e* 191 (prominent in the synthetic lactones (10–15%) but of low intensity in the natural compounds $X_{5.1}$, $X_{5.2}$ and $X_{5.3}$ (1–2%)) and *m/e* 158 (absent in the synthetic lactones but very prominent in the natural compounds $X_{5.2}$ (22%) and $X_{5.3}$ (69%)).

Taken together the GC.- and MS.- data show that the carbohydrate previously isolated from bile has a structure different from 3-*C*-hydroxymethyl-D-riburonic acid.

## Experimental Part

*3-C-hydroxymethyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose* (**3**). The nitromethyl derivative **1** (3.19 g, 10 mmol) was dissolved in 0.1 N KOH (120 ml) and the solution saturated with NaCl. Saturated NaCl solution (220 ml) and 2 M aqueous $MgSO_4$ (60 ml) was added with stirring, followed by chloroform (100 ml). After cooling in an ice-salt bath to $-12°$, a pre-cooled solution of potassium permanganate (1.45 g) in saturated NaCl solution (150 ml) was added dropwise during 20 min with vigorous stirring, the temperature being maintained below –12° throughout. Stirring was continued for a further 5 min after completing the addition. Excess sodium borohydride was added and the solution stirred for a further 20 min; a solution of sodium sulfite (2 g) in water (15 ml) was then added, followed by cautious addition of 1 N HCl until the brown colour of manganese dioxide had disappeared. The chloroform phase was separated and the aqueous phase further extracted with chloroform (3 × 100 ml). The combined organic phases were dried over $Na_2SO_4$ and evaporated to give a colourless oil (2.86 g). This was purified by column chromatography on silica gel (75 g). Short elution with benzene followed by ethyl acetate/benzene (1:4, *v/v*) removed some unreacted nitro compound (Rf 0.8 on TLC., $SiO_2$, ethyl acetate/benzene 1:1, *v/v*) and what appeared to be 1,2:5,6-di-*O*-isopropylidene-allose (Rf 0.5 on TLC.). Further elution with a gradient of ethyl acetate in benzene followed by pure ethyl acetate yielded the hydroxymethyl derivative (Rf 0.3 on TLC.) as a colourless oil which slowly crystallized (1.9 g, 6.5 mmol, 65%). – IR. ($\nu_{max}$, $CHCl_3$): 3540, 1386, 1376 $cm^{-1}$. – NMR. ($\delta$, $CDCl_3$): 5.79 (1H, *d*, $J$ = 4 Hz, H(1)); 4.59 (1H, *d*, $J$ = 4 Hz, H(2)); 1.60 and 1.47 (each 3H, *s*, *gem.* $CH_3$); 1.37 (6H, *s*, *gem.* $CH_3$). – $[\alpha]_D^{22} + 20°$ ($c$ = 1.3, $CHCl_3$).

$C_{13}H_{22}O_7$ (290) Calc. C 53.8% H 7.6% Found C 53.5% H 7.9%

*3-C-acetoxymethyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose* (**4**). The hydroxymethyl derivative **3** (1.6 g, 5.5 mmol) was acetylated with acetic anhydride (6 ml)/pyridine (3 ml) for 1 h at room temperature. After removal of excess reagents under high vacuum the acetoxymethyl derivative was isolated as an oil in quantitative yield. IR. ($\nu_{max}$, film): 3490, 1745, 1385, 1375 $cm^{-1}$. – NMR. ($\delta$, $CDCl_3$): 5.77 (1H, *d*, $J$ = 4 Hz, H(1)); 4.49 (1H, *d*, $J$ = 4 Hz, H(2)); 4.44 (1H, *d*, $J$ = 11.5 Hz, $-CH_AH_BOAc$); 4.15 (1H, *d*, $J$ = 11.5 Hz, $-CH_AH_BOAc$); 2.12 (3H, *s*, OAc); 1.61, 1.46, 1.38, 1.36 (each 3H, *s*, *gem.* $CH_3$).

*3-C-acetoxymethyl-1,2-O-isopropylidene-α-D-allofuranose* (**5**). The acetoxymethyl derivative **4** (1.45 g, 4.4 mmol) was dissolved in methanol (45 ml) and diluted with water (30 ml). 0.2 N HCl (20 ml) was then added and the solution kept at room temp for 24 h. Acid was removed by passage through a column (1.5 × 7 cm) of Amberlite IR-45 ($HCO_3^-$), and the eluate and washings (50% aqueous methanol) were evaporated to dryness under reduced pressure to give a gum (1.35 g). This material was purified by chromatography on silica gel (17 g). The column was washed with ethyl acetate/benzene (1:1, *v/v*) to remove a small amount of starting material, and the pure 5,6-diol was then eluted with ethyl acetate. Evaporation gave a colourless oil which rapidly crystallized (1.10 g, 3.8 mmol, 85%), m.p. 84–85°. IR. ($\nu_{max}$, $CHCl_3$): 3530, 3400, 1740, 1387, 1378 $cm^{-1}$. – NMR. ($\delta$, $CDCl_3$–$D_2O$): 5.72 (1H, *d*, $J$ = 4 Hz, H(1)); 4.50 and 4.07 (each 1H, *d*,

$J$ = 12 Hz, $H_A$ and $H_B$ of $-CH_2OAc$); 4.2 (1H, *d*, $J$ = 4 Hz, H(2)); 2.12 (3H, *s*, OAc); 1.57 and 1.37 (each 3H, *s*, *gem.* $CH_3$). – $[\alpha]_D^{22}$ + 20° ($c$ = 1.29, $CHCl_3$).

$C_{12}H_{20}O_8$ (292) Calc. C 49.3% H 6.9% Found C 48.7% H 7.4%

*5,3¹-hemiacetal of 3-C-hydroxymethyl-1,2-O-isopropylidene-α-D-ribo-pentodialdose* (**6**). The 5,6-diol **5** (520 mg, 1.8 mmol) was dissolved in water (15 ml) and a solution of $NaIO_4$ (520 mg) in water (15 ml) added. After 5 min at room temp. the solution was passed five times through a column of the mixed-bed ion-exchanger Amberlite MB 3 (18 g). The column was washed with water (150 ml), and the combined eluate and washings were evaporated to dryness under reduced pressure to give the hemiacetal as white flakes (330 mg, 1.5 mmol, 85%). Traces of formaldehyde were removed from the solid under high vacuum at 50°. Recrystallisation from acetone/petroleum ether gave glistening white flakes, m.p. 112–115°. IR. ($\nu_{max}$, nujol): 3365. – NMR. ($\delta$, $d_6$-DMSO): 6.13 (1H, *d*, $J$ = 4.5 Hz, OH(5), signal disappeared upon exchange with $D_2O$); 5.68 (1H, *d*, $J$ = 3.5 Hz, H(1)); 5.07 (2H, *m*, H(5) and OH(3), $D_2O$ converted signal to 1H, *s*); 4.35 (1H, *d*, $J$ = 3.5 Hz, H(2)); 4.08 (1H, *s*, H(4)); 3.94 and 3.70 (each 1H, *d*, $J$ = 9.5 Hz, $H_A$ and $H_B$ of $-CH_2-$); 1.48 and 1.30 (each 3H, *s*, *gem.* $CH_3$). – $[\alpha]_D^{22}$ + 72° ($c$ = 3.4, $CHCl_3$). – MS. (*m/e*): 203 (12.2%, $M^+ - 15$), 189 (2.9%), 171 (16.4%), 143 (6.5%), 139 (15.0%), 114 (6.8%), 101 (12.2%), 97 (17.7%), 85 (41.6%), 71 (31.4%), 59 (100.0%), 43 (83.1%).

$C_9H_{14}O_6$ (218) Calc. C 49.5% H 6.4% Found C 49.7% H 6.6%

*5,3¹-Lactone of 3-C-hydroxymethyl-1,2-O-isopropylidene-α-D-riburonic acid* (**7**). The hemiacetal **6** (100 mg, 0.46 mmol) was oxidised overnight at room temp. with anhydrous dimethyl sulfoxide (2.4 ml) and acetic anhydride (0.6 ml). Excess reagents and volatile products were removed under high vacuum to give a yellow gum, which was dissolved in benzene and chromatographed on silica gel (2.7 g). Elution with ethyl acetate/benzene (3:97, *v/v*) removed minor by-products, the required lactone being obtained by elution with ethyl acetate/benzene (6:94, *v/v*). Crystallisation from ether containing a trace of acetone gave the lactone as a white microcrystalline powder (63 mg, 0.29 mmol, 63%), m.p. 130–133°. IR. ($\nu_{max}$, $CHCl_3$): 3550, 1794 $cm^{-1}$. – NMR. ($\delta$, $CDCl_3$): 5.93 (1H, *d*, $J$ = 3.5 Hz, H(1)); 4.40 (1H, *d*, $J$ = 10 Hz, $H_A$ of $-CH_2-$); 4.37 (1H, *d*, $J$ = 3.5 Hz, H(2)); 4.27 (1H, *s*, H(4)); 4.10 (1H, *d*, $J$ = 10 Hz, $H_B$ of $-CH_2-$); 3.13 (1H, *d*, $J$ = 1.5 Hz, OH(3), signal disappeared upon exchange with $D_2O$); 1.58 and 1.38 (each 3H, *s*, *gem.* $CH_3$). – $[\alpha]_D^{22}$ + 55° ($c$ = 3.8, $CHCl_3$). – MS. (*m/e*): 202 (28.1%, $M^+ - 14$), 201 (30.6%, $M^+ - 15$), 139 (34.7%), 102 (21.8%), 101 (27.8%), 97 (13.2%), 85 (18.5%), 71 (17.2%), 59 (64.6%), 43 (100.0%), 29 (44.3%).

$C_9H_{12}O_6$ (216) Calc. C 50.0% H 5.6% Found C 50.2% H 5.8%

*3-C-hydroxymethyl-D-riburonic acid* (**10**) *and its lactones* (**8**, **9**). The isopropylidene lactone **7** was hydrolysed for 30 min at 100° with 0.01 N HCl, taken to dryness under $N_2$ at 60° and dried in a high vacuum over KOH. The resulting gum, consisting of a mixture of the compounds **8**, **9** and **10**, was trimethylsilylated with pyridine/hexamethyldisilazane/chlorotrimethylsilane (10:2:1, *v/v*) according to *Sweeley et al.* [7]. The reaction mixture was directly injected onto the column (glass, 190 cm length, 3 mm internal diameter, 2% SE-30 on Chromosorb W) of an LKB gas chromatograph/mass spectrometer type 9000 (70 eV). Conditions were as follows: column temp., 150°; injector block temp., 220°; separator temp., 250°; ion-source temp., 270°; carrier gas, He, 25 ml/min. Thus the conditions were closely similar to those used in previous analyses of the natural compounds isolated from bile [1].

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