The stability toward hydrolysis of ATP ternary complexes has been investigated by Sigel and co-workers,[27,28,29] who have proposed a structural model which accounts for the properties of these complexes in solution.[29] These investigations are of great importance for their biological implications, among which are (a) the mechanism of ATP transport in biological fluids and (b) the existence of ternary enzyme–metal–substrate complexes in enzyme-catalyzed dephosphorylation reactions. The crystal structure of $[Zn(H_2ATP)(bpy)]_2$ is essentially in agreement with Sigel's model:[29] (1) the chelating bipyridyl does not allow interaction of the metal ion with N7 of the base, which appears to be an essential step in the hydrolysis mechanism;[28] (2) the phosphate chain binds essentially through the β- and γ-phosphate groups, the α-phosphate group being only weakly bound; (3) a metal-ion-bridged intramolecular stacking adduct between the bipyridyl ligand and the purine base is present.

The structure of $[Zn(H_2ATP)]_2$ gives also support to the mechanism proposed for the transfer of the phosphoryl group catalyzed by those enzymes which use ATP as substrate, such as kinases.[29,30] The mechanism proposed, in fact, is based upon the possibility for the metal ion to shift from the β,γ phosphate coordination, to the α,β coordination. The present structure shows that this can be easily performed by a slight shortening of the Zn–O3 bond and consequent loosening of the Zn–O9 bond.

Supplementary Material Available: Table 1s, bond lengths in $[Zn(H_2ATP)(bpy)]_2$; Table 2s, bond angles in $[Zn(H_2ATP)(bpy)]_2$; Table 3s, least-squares plane equations; and observed and calculated factors (12 pages). Ordering information is given on any current masthead page.

(27) Sigel, H. *J. Am. Chem. Soc.* **1976**, *98*, 730.
(28) Sigel, H.; Buisson, D. H.; Prijis, B. *Bioinorg. Chem.* **1975**, *5*, 1.
(29) Sigel, H.; Amsler, P. E. *J. Am. Chem. Soc.* **1976**, *98*, 7390.
(30) Dunaway-Mariano, D.; Benovic, J. L.; Cleland, W. W.; Gupta, R. K.; Mildvan, A. S. *Biochemistry* **1979**, *18*, 4347.

Highly Selective *re* Additions to a Masked Oxaloacetate. Absolute Configurations of Fluorocitric Acids

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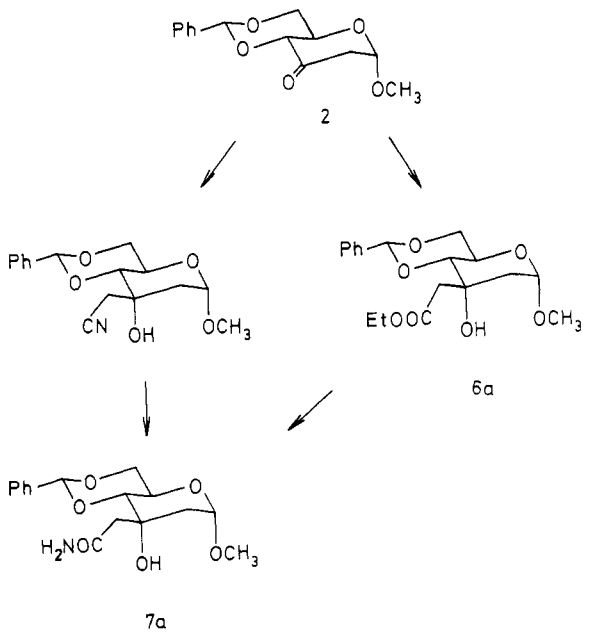
Abstract: Reformatsky reagents derived from ethyl bromofluoroacetate and ethyl 2-bromopropionate add with high selectivity (>97.5%) to the carbonyl group of **2** in a *re* (equatorial) manner, yielding **3a** + **3b** and **4a** + **4b**, respectively. The *re* stereochemistry of the former reaction was established by reductive defluorination of **3a** and **3b** to **10** with lithium triethylborohydride. Contrary to previous evidence, addition of the anion of cyanomethane to **2** also proceeds in a *re* fashion. The conformers in which the fluorine atom and the hydroxyl group at C-3 are trans predominate (>60%) in **3a** and **3b**. The Reformatsky products (e.g., **3a** and **3b**) could be degraded in a single step into substituted citric acids (e.g., **1a** and **1b**, respectively) and **2** can therefore be regarded as a masked oxaloacetate which gives highly selective *re* additions with the above reagents. Since the relative configurations of fluorocitric acids have been determined earlier, the absolute configurations of **1a** (1*R*,2*S*)[1] and **1b** (1*S*,2*S*)[1] could be assigned. The 1*R*,2*R* configuration could thus be ascribed to that isomer of fluorocitric acid which is formed in the citrate synthase reaction with fluoroacetyl-CoA.

The enzyme citrate (*si*)-synthase (EC 4.1.3.7) catalyzes the biosynthesis of citric acid from oxaloacetate and acetyl-CoA.[2] Fluoroacetyl-CoA also serves as a substrate and synthesis of fluorocitric acid[1] thus ensues.[3] A single stereoisomer of fluorocitric acid is formed[4] and this isomer is much more toxic than the other three stereoisomers. Recent evidence indicates that its main toxicity is due to an irreversible inhibition of the citrate transport in mitochondria rather than to the long-known inhibition of aconitase (EC 4.2.1.3).[5] An X-ray investigation of the racemate containing the inhibitory isomer revealed that the isomer belongs to the 1*RS*,2*RS* pair,[1,6] but the absolute configuration was not determined.[7] A biosynthesis of fluorocitric acid which proceeds with the same stereochemistry as that leading to citric acid[8] would give a fluorocitric acid with 2*R* configuration; the 1*R*,2*R* isomer (**1c**) has therefore been regarded[6a] as the probable inhibitory

Scheme I



(1) The numbering of citric acid and its derivatives is that of 2-hydroxy-1,2,3-alkanetricarboxylic acids.
(2) (a) Spector, L. B. *Enzymes* **1972**, *7*, 357–368; (b) Srere, P. A. *Adv. Enzymol.* **1975**, *43*, 57–101; (c) Weitzman, P. D. J.; Danson, M. J. *Curr. Top. Cell. Regul.* **1976**, *10*, 161–204.
(3) Fanshier, D. W.; Gottwald, L. K.; Kun, E. *J. Biol. Chem.* **1964**, *239*, 425–434.
(4) Dummel, R. J.; Kun, E. *J. Biol. Chem.* **1969**, *244*, 2966–2969.
(5) (a) Kun, E.; Kirsten, E.; Sharma, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4942–4946; (b) Kirsten, E.; Sharma, M. L.; Kun, E. *Mol. Pharmacol.* **1978**, *14*, 172–184.
(6) (a) Carrell, H. L.; Glusker, J. P.; Villafranca, J. J.; Mildvan, A. S.; Dummel, R. J.; Kun, E. *Science* **1970**, *170*, 1412–1414; (b) Carrell, H. L.; Glusker, J. P. *Acta Crystallogr., Sect. B* **1973**, *29*, 674–682.
(7) An X-ray investigation which appeared after the completion of our manuscript shows that the inhibitory isomer possesses the 1*R*,2*R* configuration: Stallings, W. C.; Monti, C. T.; Belvedere, J. F.; Preston, R. K.; Glusker, J. P. *Arch. Biochem. Biophys.* **1980**, *203*, 65–72.

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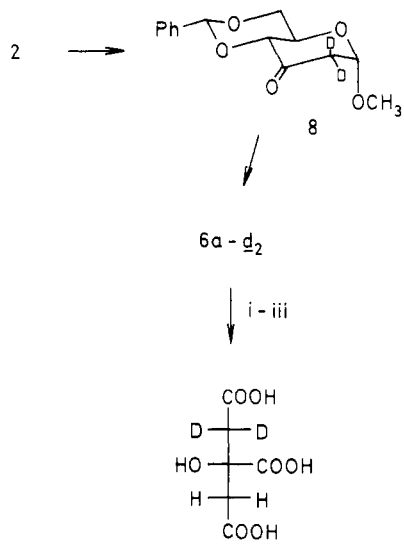
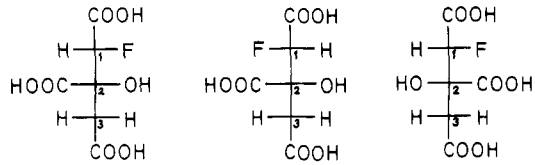
Scheme II

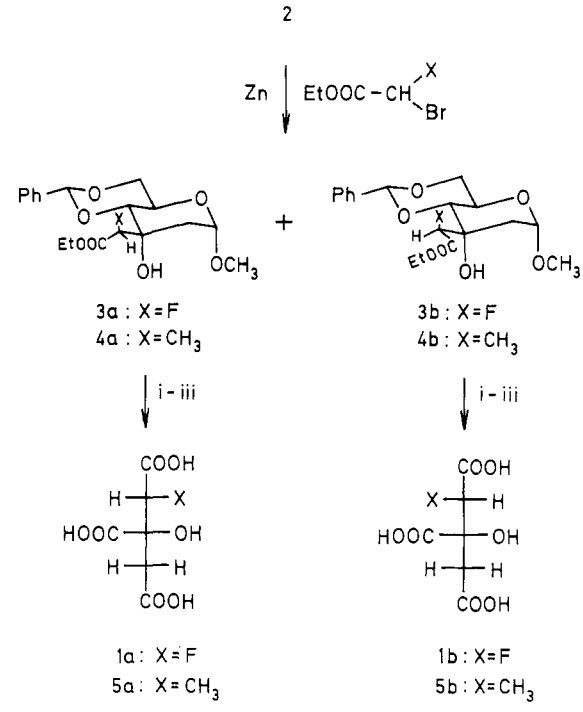


isomer. We report herein an enantioselective synthesis of **1b** which allows the unequivocal assignment of the 1*R*,2*R* configuration (**1c**) to the inhibitory isomer of fluorocitric acid.



A stereocontrolled synthesis of only one of the enantiomers of the 1*RS*,2*RS* pair was conceptualized along the lines of a citrate biomimetic synthesis. Thus, we sought a masked oxaloacetate which, like the citrate synthase-bound oxaloacetate, would allow nucleophilic attack at the keto carbonyl group from only one of its diastereotopic faces. Methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose[13] (**2**) seemed to fulfill this requirement. For ketone **2** the conformer shown (Scheme I) is expected to be strongly preponderant. In this conformer a *re* (equatorial) attack on the C-3 keto group[14] should be unhindered, whereas a *si* (axial) attack should be strongly hindered by the unfavorable ring puckering and by the axial methoxy group at C-1. For the demasking of the oxaloacetate carboxylic groups, an oxidative degradation involving glycol cleavage of the C-(4)–C(5) bond was envisaged.

Scheme III



Previous Additions to the Keto Group of 2

Several nucleophilic additions to the carbonyl group of **2** have been found to proceed with a strong preference for equatorial (*re*) attack.[15-19] However, the anion of cyanomethane was reported to add to the carbonyl group exclusively in the axial mode.[20] We reinvestigated the reaction of **2** and cyanomethane and confirmed that a single product is formed. The nitrile was converted into the corresponding amide (**7a**) by using hydrogen peroxide in alkaline solution.

Reaction of **2** with the Reformatsky reagent from ethyl bromoacetate gave two products in 94:6 ratio (**6a**/**6b**). Both esters were transformed into the corresponding amides via the hydrazides, and the major one was identical with the amide from the cyanomethane route (Scheme I). Thus, the steric course of the Reformatsky reaction is identical with that of the cyanomethane reaction. The direction of the steric course was determined as shown in Scheme II. Treatment of **2** with D_2O yielded the dideuterio ketone **8**, which was allowed to react with ethyl bromoacetate and zinc. The major Reformatsky product was degraded into (-)-[1,1-$^2H_2$]citric acid by using the permanganate method described below. This enantiomer must have the *S* configuration since it has previously been found to retain[21] all the deuterium label on reaction with a tissue preparation containing aconitase, which is known[22] to react with the (*pro-R*)-carboxymethyl group of citric acid. Consequently, the major product is formed by addition of the Reformatsky reagent to the keto group from the equatorial side. This means that the configuration at

(8) Contrary to the interpretation by Hanson[9] and numerous later authors, the results of Hanson and Rose[10] do not unequivocally demonstrate a *si* attack on oxaloacetate. A *re* attack, followed by ejection of CoA by a β-lactone-forming process and finally ring opening with inversion of configuration by attack of water at the oxygen-bearing, sp^3-hybridized ring carbon, would also lead to the incorporation of the acetyl carbons into the (*pro-S*)-carboxymethyl group of citric acid. However, this latter possibility is excluded by the later finding[11] that, when running the reaction in $H_2{}^{18}O$, ^{18}O enters the terminal carboxyl group in the citric acid rather than the alcoholic hydroxyl group. The possible intermediacy of a β-lactone was first discussed by Cornforth and by Eschenmoser and Arigoni.[12]

(9) Hanson, K. R. *J. Am. Chem. Soc.* **1966**, *88*, 2731–2742.

(10) Hanson, K. R.; Rose, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 981–988.

(11) Suelter, C. H.; Arrington, S. *Biochim. Biophys. Acta* **1967**, *141*, 423–425.

(12) (a) Cornforth, J. W. *J. Lipid Res.* **1959**, *1*, 3–28; (b) Eschenmoser, A.; Arigoni, D., personal communication referred to by Lynen, F. *J. Cell. Comp. Physiol., Suppl. 1* **1959**, *54* 33–49.

(13) Klemer, A.; Rodemeyer, G. *Chem. Ber.* **1974**, *107*, 2612–2614.

(14) The carbohydrate numbering has been retained throughout.

(15) (a) Howarth, G. B.; Jones, J. K. N. *Can. J. Chem.* **1967**, *45*, 2253–2256; (b) Dyong, I.; Schulte, G. *Tetrahedron Lett.* **1980**, *21*, 603–606.

(16) Sepulchre, A. M.; Septe. B.; Lukacs, G.; Gero, S. D.; Voelter, W.; Breitmaier, E. *Tetrahedron* **1974**, *30*, 905–915.

(17) Yoshimura, J.; Matsuzawa, M.; Funabashi, M. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2064–2067.

(18) Rosenthal, A.; Ong, K.-S. *Can. J. Chem.* **1970**, *48*, 3034–3038.

(19) We reinvestigated the reaction with nitromethane and found that under the reaction conditions, the major isomer (63% isolated yield[18]) was slowly converted into the minor isomer (22% isolated yield[18]). After 21 and 48 h, the isomer ratio was determined by ^1H NMR and approximate conversions of 21% and 35%, respectively, were noted. Thus, the isomer ratio reported[18] reflects both kinetic and thermodynamic control.

(20) Rosenthal, A.; Schöllnhammer, G. *Can. J. Chem.* **1974**, *52*, 51–54.

(21) Martius, C.; Schorre, G. *Justus Liebigs Ann. Chem.* **1950**, *570*, 143–147.

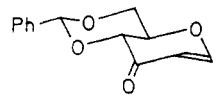
(22) Glusker, J. P. *Enzymes* **1971**, *5*, 413–439.

C-3 previously ascribed[20] to the cyanomethane reaction product must be reversed.

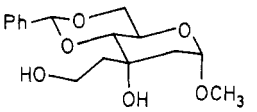
Synthesis of Fluorocitric Acids

For the synthesis of fluorocitric acids, a Reformatsky reaction between **2** and ethyl bromofluoroacetate was applied (Scheme III).[23] The Reformatsky reagent obtained from this ester had previously been generated and allowed to react with cyclohexanone in refluxing toluene–xylene.[24] The literature procedure when applied to **2** gave extensive degradation to the α,β-unsaturated ketone **9**[25] and only negligible yields of the desired Reformatsky products. An experimental modification, which included the use of specially activated zinc in tetrahydrofuran, proved satisfactory. By this procedure the amount of **9** formed was less than 5% and two major products **3a** and **3b** were obtained in yields of 23% and 63%, respectively.[26] Only one C-3 epimer of **3a** or **3b** was detected (1.4% yield); the other must amount to 0.2% or less. After chromatography on silica gel and crystallizations, the desired esters **3a** and **3b** were obtained in 14% and 47% yield, respectively.

The key step in the synthetic sequence was the degradation of the Reformatsky products **3a** and **3b** to fluorocitric acids. This step corresponds to a demasking of the oxaloacetate carboxyl groups and it was effectuated by acidic hydrolysis of the acetal functions in **3a** and **3b** and subsequent oxidation with potassium permanganate under alkaline conditions (0.13 M NaOH, 23 °C, 15 h). Acidic conditions in the oxidation step were unsuitable, probably because of further oxidation of the α-hydroxy carboxylic acid moiety. Both **3a** and **3b** on oxidative degradation gave a fluorocitric acid in about 30% yield. The optical rotations, ^{1}H NMR spectra, and GLC of trimethyl esters established that the two fluorocitric acids were diastereomers. By treatment of one of the trimethyl esters (**1a**) with sodium methoxide in methanol, a partial conversion into **1b** was achieved and it was thus evident that the two fluorocitric acids differed in their configurations at C-1, the fluorine-bearing carbon. This means that the Reformatsky reagent derived from ethyl bromofluoroacetate selectively (>98%) had attacked the C-3 keto group of **2** at one of its diastereotopic faces. A comparison with the Reformatsky reactions of **2** with ethyl bromoacetate, for which a selectivity for *re* attack of 94% was noted, and with ethyl bromopropionate, for which the *re* selectivity exceeds 97.5% (see below), leads to the conclusion that, in all probability, **3a** and **3b** are formed by a *re* (equatorial) attack on the keto group of **2**. Compelling evidence for this stereochemistry was obtained by correlating **3a** and **3b** with **6a**. Thus reduction of **6a** with lithium aluminum hydride and **3a** and **3b** with lithium triethylborohydride (56% yield)[27] yielded the same primary alcohol (**10**). The configurations of the two fluorocitric



9



10

acids obtained on degradation are thus 1*R*,2*S* (**1a**) and 1*S*,2*S* (**1b**). To the best of our knowledge, no reductive cleavage of the carbon–fluorine bond with lithium triethylborohydride has previously been described in the literature.[28,29] In view of the high stability of the C–F bond, the present reduction represents a significant extension of the reducing ability of this hydride reagent.

In order to disinguish between the 1*R*,2*S* and 1*S*,2*S* diastereomers of fluorocitric acid, we compared the trimethyl esters of the acids by GLC and ^{1}H NMR with the isomer of fluorocitric acid formed in the citrate synthase reaction with fluoroacetyl-CoA. The trimethyl esters belonging to the 1*RS*,2*RS* pair were thus found to have longer retention times on two columns than the esters belonging to the 1*RS*,2*SR* pair. The characteristic differences in the ^{1}H NMR spectra of the diastereometric esters[4] could also be used. Our synthetic fluorocitric acid belonging to the 1*RS*,2*RS* pair, i.e., the 1*S*,2*S* isomer (**1b**), showed $[\alpha]_D$ + 11.0°; the diastereomer (**1a**) showed $[\alpha]_D$ +20.1°. The *noninhibitory* enantiomer was reported[4] to have $[\alpha]_D$ +12.4°, which is why the absolute configuration of the inhibitory (levorotatory[4]) enantiomer must be 1*R*,2*R* (**1c**). Consequently, on the basis of the absolute configuration, the biosynthesis of the inhibitory isomer of fluorocitric acid is analogous to that of citric acid.

The *re* addition to the masked oxaloacetate **2** corresponds to a *re* addition to oxaloacetate itself and, in this respect, the present synthetic sequence mimics the reaction of citrate (*re*)-synthase,[30] an enzyme found in a few anaerobic bacteria, rather than that of citrate (*si*)-synthase, the mammalian enzyme.

Synthesis of Methylcitric Acids

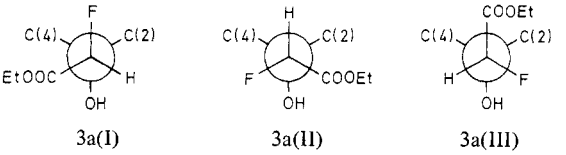
Since the absolute configurations of all methylcitric acids are known,[31] it is possible to ascertain the steric course of the reaction between **2** and the Reformatsky reagent derived from ethyl bromopropionate. The major products **4a** (42% yield) and **4b** (51%)[26] were degraded with permanganate to yield (2*R*,3*R*)- and (2*R*,3*S*)-methylcitric acid,[31] respectively. Only one C-3 epimer of **4a** or **4b** was found (2.1%), the *re* selectivity thus being 97.8%.

Conformational Aspects

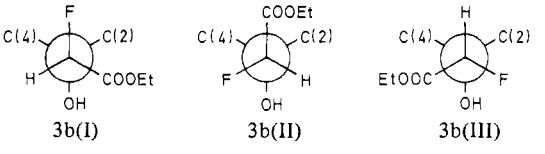
From the configurations and the spectral properties of the Reformatsky products **3a** and **3b**, it is possible to draw conclusions about their preferred conformations. Analyses[32] of the ^{1}H and ^{13}C NMR spectra of **3a** and **3b** gave the relative populations of the staggered conformers **3a**(I–III) and **3b**(I–III) (Scheme IV). The conformers **3a**(I) and **3b**(I), in which the fluorine atom and the C-3 hydroxyl group are trans, were found to predominate (relative populations >60%).

In the ^{1}H NMR spectrum of **3a**, the protons of the ethyl group give signals at δ 3.82 (CH_2) and 1.02 (CH_3). Selective removal of the benzylidene group caused a shift of the signals to normal values (δ 4.29 and 1.33). This indicates a significant contribution from conformer **3a**(I), in which the ethyl group projects into the

Scheme IV. Staggered Conformers of the Reformatsky Products **3a** and **3b**. The Trans Conformers **3a(I)** and **3b(I)** Predominate (relative populations, >60%)



3a(I) 3a(II) 3a(III)



3b(I) 3b(II) 3b(III)

(23) We were unable to generate a lithium ester enolate from ethyl fluoroacetate and lithium diisopropylamide (THF, −78 to 20 °C).

(24) McBee, E. T.; Pierce, O. R.; Christman, D. L. *J. Am. Chem. Soc.* **1955**, *77*, 1581–1583.

(25) Collins, P. M. *Carbohydr. Res.* **1969**, *11*, 125–128.

(26) Yields are as found by capillary column GLC on the crude reaction mixture.

(27) Small amounts of **10** were possibly traced by TLC after reduction of **3a** or **3b** with lithium aluminum hydride.

(28) Brown, H. C.; Kim, S. C.; Krishnamurthy, S. *J. Org. Chem.* **1980**, *45*, 1–12; Krishnamurthy, S.; Brown, H. C. *J. Org. Chem.* **1980**, *45*, 849–856.

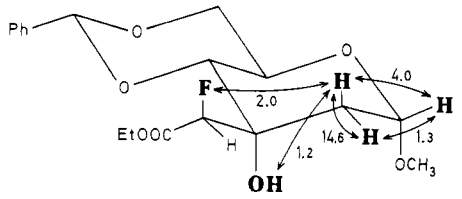
(29) Reduction with lithium aluminum hydride seems only to occur for specially activated compounds or under forcing conditions: (a) Forche, E. (*Houben-Weyl*) *Meth. Org. Chem.* **1962**, *3*, 498–499; (b) Pizey, J. S. "Synthetic Reagents", Wiley: New York, 1974; Vol. I, pp 244–247; (c) Hudlický, M., "Chemistry of Organic Fluorine Compounds", 2nd ed.; Wiley: New York, 1976; pp 183–188; (d) Pinder, A. R., *Synthesis* **1980**, 425–452.

(30) Reference 2a, pp 363–364.

(31) One of the initially reported absolute configurations has recently been revised: (a) Brandänge, S.; Josephson, S.; Mörch, L.; Vallén, S. *Acta Chem. Scand., Ser. B* **1977**, *31*, 307–312; (b) Brandänge, S.; Dahlman, O.; Mörch, L., *J. Chem. Soc., Chem. Commun.* **1980**, 555–556.

(32) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A., "Conformational Analysis"; Wiley: New York, 1965; pp 152–156; Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; pp 420–433; Thomas, W. A. *Ann. Rep. NMR Spectrosc.* **1970**, *3*, 91–147.

Scheme V. Coupling Constants (in hertz) Involving H-2-ax and H-2-eq in Compound **3a**. The Values Obtained for **3b** Are Similar Except That No Coupling to the Hydroxylic Proton Was Seen



shielding zone of the benzene ring.[33,34] The dependence of the position of the methyl signal on temperature was studied in the interval −50 to 50 °C, and a maximum shielding corresponding to about δ 0.95 was estimated by S curve extrapolation.[35] If it is assumed that this value corresponds to the trans conformer **3a**(I) and that δ 1.33 corresponds to the two gauche conformers **3a**(II) and **3a**(III), then the ^{1}H NMR results for **3a** indicate that the relative population of **3a**(I) is approximately 80% at room temperature.[36] In the ^{13}C NMR spectrum of **3a**, unequal three-bond couplings to fluorine were recorded for C-2 ($^3J_{CF}$ = 3.3 Hz) and C-4 ($^3J_{CF} \lesssim 0.5$ Hz). Although relatively few experimental data demonstrating angular dependence of this type of coupling are available, it seems clear that a trans coupling of 11–12 Hz represents the maximum value in unstrained aliphatic systems[37] and that the gauche coupling is considerably weaker. Most values reported for the latter are less than 1 Hz but two exceptions can be found in the literature.[38] Since the fluorine coupling to C-4 was below the detection limit, the gauche coupling to C(4) in the major conformer **3a**(I) must be small and the percentage of conformer **3a**(III), in which fluorine and C(4) are trans, must be negligible (≲5%). If one assumes gauche couplings in **3a**(I) of $J < 0.5$ Hz, one finds from the fluorine coupling to C(2) that the relative population of **3a**(II) is between 25% and 30%. These data in conjunction with those of the ^{1}H NMR study indicate that the conformers **3a**(I) and **3a**(II) have relative populations of about 75% and 25%, respectively.

In compound **3b** the ethyl protons give NMR signals at δ 4.26 (CH_2) and 1.29 (CH_3); $^3J_{C(2)\text{-}F}$ = 1.4 Hz and $^3J_{C(4)\text{-}F}$ = 1.1 Hz. A calculation based on the same approximations as above[39] gives relative populations of **3b**(I) and **3b**(III) of approximately 80% and 10%, respectively. However, in view of the uncertainties mentioned above, we conclude that the relative populations of **3a**(I) and **3b**(I) exceed 60%.

Some support for the above conclusions may be adduced from the long-range spin couplings between fluorine and H-2 of **3a** and **3b**. In both compounds only the low-field H-2 displays a coupling to fluorine ($^4J_{HF}$ = 2.0 Hz) and this indicates that the orientations of fluorine in **3a** and **3b** are similar. Fluorine decoupling gave a sharpening of the signals from the high-field H-2 in the two compounds but no splitting was seen at 100 MHz. Since the couplings between H-1 and the two H-2 (Scheme V) are practically identical with the well-defined corresponding couplings in methyl 2-deoxy-α-D-*ribo*-hexopyranoside,[40] the low-field H-2 in **3a** and **3b** could be assigned as H-2-ax and the high-field one as H-2-eq.[41] This means that the fluorine atoms of **3a** and **3b** apparently couple selectively to the axial H-2. This selectivity is consistent with a strongly preferred trans disposition of the fluorine atom in both **3a** and **3b** relative to the C-3 hydroxyl group. Long-range coupling constants $^4J_{HF}$ in 1-fluoropropane have been calculated for various spatial arrangements of the coupling nuclei.[44] For the arrangements corresponding to the **3a**(I) and **3b**(I) conformations (Scheme IV), the calculation predicts values for $^4J_{HF}$ of −2.63 and −0.86 Hz for the couplings to H-2-ax and H-2-eq, respectively. Although only few experimental data are available to confirm the relevance of this calculation, it probably supports the interpretation that **3a**(I) and **3b**(I) are major conformations.

It should be pointed out that the conformational preferences of **3a** and **3b** are not analogous to those of simple fluorohydrins such as 2-fluoroethanol or 1-fluoro-2-propanol. The latter compounds show a strong preference for adopting gauche conformations (>85%).[45]

Experimental Section

THF was distilled over $LiAlH_4$ before use. Lithium triethylborohydride was purchased from Aldrich as a 1 M solution in THF. Analytical GLC was performed either on a packed column (3% JXR on Gas-Chrom Q, 100–120 mesh, 2.4 m) or on a SP-2100 fused silica capillary column (25 m). Compositions of Reformatsky reaction mixtures were calculated by electronic integration of all peaks, assuming equal response factors for all compounds. Fine separations were carried out on four Lobar Fertigsäule columns linked in a series (LiChroprep Si 60, 40–63 μm, 25 × 310 mm, Merck). Preparative HPLC of trimethyl esters of **1a**, **1b**, **5a**, and **5b** was carried out on a silica gel column (Partisil 10, Reeve Angel, 25 cm × 4.6 mm), eluting with 2,2,4-trimethylpentane/ethyl acetate (5:2). ^{1}H and ^{13}C NMR chemical shifts are relative to internal Me_4Si in $CDCl_3$ or sodium 2,2-dimethyl-2-silapentane-5-sulfonate in D_2O. The three-bond coupling constants F–C–C–C(2) and F–C–C–C(4) in **3a** and **3b** were obtained by measuring ^{13}C NMR spectra at 50 MHz (33 °C) with a Bruker WP 200 instrument, the limiting resolution being that of the spectrometer (≥0.07 Hz). Couplings to the H-2 protons of **3a** and **3b** were studied under fluorine decoupling on a Varian XL-100 spectrometer, the limiting resolution being ≥0.25 Hz. Other NMR data were obtained on a JEOL JNM-FX 100 instrument (digital resolutions of 0.24 and 0.61 Hz in ^{1}H and ^{13}C NMR, respectively; spectrometer resolution ≥0.3 Hz). The assignments of ^{13}C signals are based on multiplicities obtained in single-frequency off-resonance spectra and on previous assignments for similar compounds.[46] IR spectra were recorded on a Perkin-Elmer 257 instrument (2-mm cell, 0.01

(33) Jackman, L. M.; Sternhell, S. "Applications of Nuclear Magnetic Resonance in Organic Chemistry"; Pergamon Press: Oxford, 1972; pp 94–98.

(34) Such a shielding requires that the benzene ring be near perpendicular to the O(4)–C(5)–O(6) plane. This arrangement has been found in the crystalline state of one 4,6-benzylidene acetal: Davison, B. E.; McPhail, A. T. *J. Chem. Soc. B* **1970**, 660–666 but not of another: Pilotti, A.-M.; Stensland, B., *Acta Crystallogr. Sect. B* **1972**, *28*, 2821–2825. In the latter case, the normals to the two planes form a positive angle of 32° with each other, which means that a more flat conformation is adopted. We thank Mr. Jan-Eric Berg for calculating this angle which is not available from the publication.

(35) The chemical shifts recorded at 50, 25, −20, and −50 °C were δ 1.041, 1.024, 1.006, and 1.011, respectively. These values must, however, be corrected for the general temperature effect (relative to Me_4Si), which was taken as the mean of the chemical shift differences Δδ observed for the OCH_3 protons and the $CHCl_3$ (except for 50 °C) proton. After this correction the positions of the CCH_3 signal were δ 1.050, 1.024, 0.987, and 0.973.

(36) This treatment neglects the temperature effect on the orientaton of the phenyl ring and on rotations around other single bonds in the C-3 side chain.

(37) (a) Schneider, H.-J.; Gschwendtner, W.; Heiske, D.; Hoppen, D.; Thomas, F. *Tetrahedron*, **1977**, *33*, 1769–1773, and references given therein; (b) Holland, H. L. *Tetrahedron Lett.* **1978**, *19*, 881–882; (c) Wray, V. *J. Chem. Soc., Perkin Trans 2* **1976**, 1598–1605.

(38) For the fluorine coupling to C-6 in 4-deoxy-4-fluoro-D-galactose, $^3J_{CF}$ = 5.5 Hz (ref 37c); for the fluorine coupling to C-10 in 3β, 5α-dihydroxy-6β-fluoroandrostan-17-one, $^3J_{CF}$ = 1.8 Hz (ref 37b).

(39) For **3b** the value δ 0.95 is a more uncertain approximation than for **3a**.

(40) Lemieux, R. U.; Levine, S. *Can. J. Chem.* **1964**, *42*, 1473–1480.

(41) Consistent with this assignment is the remarkable long-range coupling which occurs between the low-field H-2 proton and the hydroxylic proton in **3a** ($^4J_{HH}$ = 1.2 Hz) but not in **3b**. The coupling disappeared when the OH proton was exchanged against deuterium by shaking with D_2O. In order to account for this coupling, a planar "W" arrangement of H–O–C(3)–C(2)–H(2) probably must be invoked.[42] Of the two H-2 hydrogens only H-2-ax can fulfill this requirement; the torsion angle H–O–C(3)–C(2) then being approximately 180°. A hydrogen bond involving the ester group seems to hold the hydroxylic proton in the required position. Accordingly, the ester group gives rise to two carbonyl stretching bands, 1768 and 1731 cm^{-1}, approximate area ratio 3:2. For **3b** only one band is seen (1755 cm^{-1}). The value obtained for **3a** indicates that the OH group of this compound is preferably hydrogen bonded to the alkyl oxygen of the ester group (cf. ref 43, p 17), and this means that the carbonyl moiety must be near eclipsed to the fluorine atom. This latter arrangement has in fact been found to be predominant in ethyl fluoroacetate: Brown, T. L., *Spectrochim. Acta* **1962**, *18*, 1615–1623.

(42) A similar long-range coupling to a hydrogen-bonded OH has been reported: Kingsburg, C. A.; Egan, R. S.; Perun, T. J. *J. Org. Chem.* **1970**, *35*, 2913–2918.

(43) Aaron, H. S. *Top. Stereochem.* **1979**, *11*, 1–52.

(44) Wasylishen, R. E.; Barfield, M. *J. Am. Chem. Soc.* **1975**, *97*, 4545–4552.

(45) Hagen, K.; Hedberg, K. *J. Am. Chem. Soc.* **1973**, *95*, 8263–8266; Marstokk, K.-M.; Møllendal, H. *J. Mol. Struct.* **1977**, *40*, 1–11.

(46) Breitmaier, E.; Voelter, W. "^{13}C NMR Spectroscopy"; Verlag Chemie: Weinheim/Bergstr., Germany, 1978; pp 250–251.

M solutions in CCl_4). Optical rotations were measured on a Perkin-Elmer 241 polarimeter and CD spectra on a Cary 60 spectropolarimeter. The CD samples (pH ≈3.1) were approximately 3 and 6 mM with respect to hydroxy acid and sodium molybdate (VI), respectively.[47]

Methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranoside-3-ulose (2) was prepared according to a modification[48] of the Klemer and Rodemeyer synthesis.[13]

Activation of Zinc. Zinc dust (70 g) was added to hydrochloric acid (3 M, 400 mL) and the mixture was allowed to react for 5–10 min without stirring. The acid was decanted, and the product was washed with deionized water (2 × 300 mL) and absolute ethanol (3 × 100 mL). The product was then filtered on a Büchner funnel and washed with dry ether (5 × 50 mL). During these treatments the zinc was kept covered with liquid and only after the last washing was it sucked dry (1 min). Contrary to other published procedures for the activation, the product was used immediately.

Reformatsky Reaction with Ethyl Bromofluoroacetate. A mixture of activated zinc dust (1.92 g, 30 mmol), ketone **2** (3.96 g, 15 mmol), ethyl bromofluoroacetate[49] (3.05 g, 16.5 mmol) in THF (60 mL) was heated. The reaction started when, or sometimes before, the boiling point was reached. After the reaction mixture was refluxed for 10 min, it was poured into sodium dihydrogen phosphate buffer (0.1 M, 400 mL) and ether (200 mL). Filtration, extractions with ether (2 × 200 mL), drying (Na_2SO_4), and concentration gave a product mixture which contained several compounds (see below). Chromatography on silica gel (5 × 65 cm, 0.040–0.063 mm) with methylene chloride/ethyl acetate (9:1) as eluant gave a practically complete separation of **3a** and **3b** (elution order: **9**, **3b**, **3a**). Compound **3b** was rechromatographed on silica gel in order to remove **9** (eluant: ethyl acetate/2,2,4-trimethylpentane, 3:2).

Methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-[(*R*)-(ethoxycarbonyl)fluoromethyl]-α-D-*ribo*-hexopyranoside (3a) was recrystallized (one crop) from ethanol: needles (0.77 g, 14%), mp 139.5–140.5 °C; $[\alpha]_D^{25}$ + 63° (*c* 1.0, ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (m, 5 H), 5.56 (s, PhCH), 4.84 (X part of ABX spectrum, $J_{AX} \approx 4.0$ Hz, $J_{BX} \approx 1.0$ Hz), 4.65 (d, ^2J = 47.1 Hz, CHF), 4.4–3.5 (m, 7 H, including an OH signal of unknown multiplicity at δ 3.83 and an OCH_2 quartet at δ 3.86), 3.42 (s, OCH_3); δ_A 2.22 and δ_B 2.07 (AB part of ABX spectrum, proton A being further coupled both to fluorine $^4J_{AF}$ = 2.0 Hz, and to OH, ^4J = 1.2 Hz, J_{AB} = 14.6 Hz, $J_{AX} \approx 4.0$ Hz, J_{BX} = 1.3 Hz), 1.02 (t, CCH_3); ^{13}C NMR ($CDCl_3$) 167.70 (d, $^2J_{CF}$ = 24.4 Hz; C=O), 137.03, 129.09, 128.01, and 126.24 (aromatic carbons), 101.87 (PhC), 98.21 (C-1), 88.15 (d, $^1J_{CF}$ = 194.1 Hz, CHF), 77.19 (C-4), 71.46 (d, $^2J_{CF}$ = 19.5 Hz, C-3), 69.17 (C-6), 61.74 ($COOCH_2$), 58.65 (C-5), 55.53 (OCH_3), 36.19 (d, $^3J_{CF}$ = 3.3 Hz, C-2), and 13.64 (CCH_3). Anal. Calcd for $C_{18}H_{23}FO_7$: C, 58.37; H, 6.26; F, 5.13. Found: C, 58.36; H, 6.20; F, 5.19.

Methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-[(*S*)-(ethoxycarbonyl)fluoromethyl]-α-D-*ribo*-hexopyranoside (3b) was recrystallized (one crop) from ethanol: prisms (2.59 g, 47%), mp 131–131.5 °C; $[\alpha]_D^{25}$ +123° (*c* 1.0, ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (m, 5 H), 5.63 (s, PhCH), 5.02 (d, ^2J = 47.9 Hz, CHF), 4.87 (broad d, $J \approx 3.9$ Hz, H-1), 4.6–3.2 (m, 10 H, including a OH doublet at δ 3.52, $^4J_{HF}$ = 2.0 Hz, a OCH_3 singlet at δ 3.41, and a OCH_2 quartet at δ 4.26), δ_A 2.21 and δ_B 2.03 (AB part of ABX spectrum, further coupled to fluorine, J_{AB} = 14.9 Hz, J_{AX} = 3.9 Hz, J_{BX} = 1.2 Hz, $^4J_{AF}$ = 2.0 Hz, $^4J_{BF} \approx 0$ Hz), 1.29 (t, 3 H); ^{13}C NMR ($CDCl_3$) 167.47 (d, $^2J_{CF}$ = 23.8 Hz, C=O), 137.15, 129.01, 128.09, and 126.24 (aromatic carbons), 101.85 (Ph-C), 98.41 (C-1), 86.18 (d, $^1J_{CF}$ = 186.8 Hz, CHF), 77.36 (d, $^3J_{CF}$ = 1.1 Hz, C-4), 70.97 (d, $^2J_{CF}$ = 22.0 Hz, C-3), 69.05 (C-6), 61.52 ($COOCH_2$), 58.87 (C-5), 55.33 (OCH_3), 33.84 (d, $^3J_{CF}$ = 1.4 Hz, C-2), and 14.08 (CCH_3). Anal. Calcd. for $C_{18}H_{23}FO_7$: C, 58.37; H, 6.26; F, 5.13. Found: C, 58.40; H, 6.24; F, 5.23.

Enone 9. After four recrystallizations from 2-propanol the compound still contained an impurity and showed mp 127.5–129.5 °C; $[\alpha]_D^{20}$ +171° (*c* 1.0, $CHCl_3$) [lit.[25] mp 128–129 °C; $[\alpha]_D$ +189° ($CHCl_3$)]. The ^{1}H NMR spectrum (100 MHz) showed good agreement with the literature[25] values (60 MHz).

Eight other minor products were isolated by chromatography on silica gel and characterized by ^{1}H NMR spectroscopy. The yields and retention times given below are as found by capillary column GLC: enone **9** (0.8%, 6.39 min), ketone **2** (4.1%, 8.20 min), four Reformatsky products derived from **9** (0.5%, 10.00 and 10.09 min; 0.2%, 10.93 min; 0.8%, 11.45 min), C-3 epimer of **3a** or **3b** (1.4%, 12.80 min), unknown (0.2%, 13.55 min), methyl ester analogue of **3a** (0.2%, 13.87 min), methyl ester analogue of **3b** (3.2%, 14.90 min), **3a** (22.5%, 15.60 min), **6a** (?, not isolated, 1.9%, 16.39 min), **3b** (63.4%, 17.28 min), ethyl glycoside analogue of **3b** (0.3%, 18.70 min).

Reduction of 3a with Lithium Triethylborohydride. A solution of **3a** (132 mg, 0.36 mmol) and lithium triethylborohydride (7 mmol) in THF (9 mL) was allowed to stand for 24 h (22 °C). After addition of water (1 mL), 2 M NaOH (1 mL), and 35% hydrogen peroxide (1 mL), the mixture was stirred for 45 min and poured into hydrochloric acid (0.5 M, 30 mL)/ice. Extraction with methylene chloride (3 × 25 mL), washing of the combined organic layers with a saturated solution of $NaHCO_3$, drying (Na_2SO_4), and evaporation gave a crude product, which was purified on silica gel with ethyl acetate/methanol/pyridine (25:1:0.025) as solvent. **Methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-(2′-hydroxyethyl)-α-D-*ribo*-hexopyranoside (10)** (62 mg, 56%) was obtained in crystalline form and was recrystallized from ethyl acetate/2,2,4-trimethylpentane: mp 128–135 °C; $[\alpha]_D^{20}$ + 95° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.6-7.2 (m, 5 H), 5.61 (s, PhCH), 4.85 (broad d, $J \approx$ 3.2 Hz, H-1), 4.4–3.2 (m, 10 H, including an OCH_3 singlet at 3.42 and an OH signal, δ ≈3.87, exchangeable in D_2O), 3.0–2.8 (OH, exchangeable in D_2O), 2.4–2.0 (m, 2 H, overlapping low-field parts of two AB spectra), 1.92, 1.88, 1.77, and 1.74 (upfield AB part of ABX spectrum, H-2), 1.70–1.4 (1 H, upfield AB part of ABX_2 spectrum, α proton of β-hydroxyethyl chain).

Reduction of **3b** as above gave the same product.

Degradation of 3a and 3b to Fluorocitric Acids. A solution of **3b** (783 mg, 2.1 mmol) in a mixture of acetic acid (13 mL) and water (67 mL) was refluxed for 30 min. The solvents were evaporated, the residue was dissolved in aqueous NaOH (0.5 M, 60 mL), and a solution of potassium permanganate (30 mmol) in water (155 mL) was then added. After 15 h at 22 °C, acetone (10 mL) was added and the mixture was stirred (5 min). Filtration, treatment with ion exchanger (Dowex 50W-X8, H^+, 4 × 20 cm), and concentration to dryness gave a residue, which was treated with ethereal diazomethane. Drying ($MgSO_4$), filtration, and evaporation of the solvent gave a crude product (392 mg), which according to a GLC determination (comparing response with that of a standard solution) contained 150 mg of trimethyl fluorocitrate (28% yield). Purification by preparative HPLC yielded the ester as a colorless liquid, which was >99% pure (GLC, HPLC). The isomeric trimethyl fluorocitrate was obtained analogously from **3a** (33% yield).

Trimethyl Ester of 1a: $[\alpha]_D^{20}$ +18.4° (*c* 2.0, methanol); ^{1}H NMR ($CDCl_3$) δ 5.03 (d, $^2J_{HF}$ = 46.9 Hz), 4.01 (s, 1 H), 3.89 (s, 3 H), 3.86 (s, 3 H), 3.70 (s, 3H), 3.07 (s, 2 H); mass spectrum, *m/e* (rel intensity) 193 (12), 175 (6), 161 (100), 133 (23), 129 (20), 101 (38), 59 (56).

Trimethyl Ester of 1b: $[\alpha]_D^{20}$ +13.7° (*c* 2.0, methanol); ^{1}H NMR ($CDCl_3$) δ 5.12 (d, $^2J_{HF}$ = 47.1 Hz), 3.92 (s, 1 H), 3.88 (s, 3 H), 3.82 (s, 3 H), 3.71 (s, 3 H), 3.04 (s, 2 H); mass spectrum, *m/e* (rel intensity) 193 (10), 175 (27), 161 (100), 147 (12), 133 (22), 129 (19), 101 (36), 59 (68).

Hydrolysis of the esters of **1a** and **1b** was carried out with aqueous NaOH (0.5 M, 23 °C, 4 days). Ion exchange on a Dowex 50W-X8 (H^+) column, evaporation of the solvent, and drying over P_2O_5 (15 h) under reduced pressure gave the fluorocitric acids which were >98% pure (^{1}H NMR).

1a: $[\alpha]_D^{20}$ + 20.1° (*c* 2.0, water); ^{1}H NMR (D_2O, 85 °C) δ 5.20 (d, $^2J_{HF}$ = 47 Hz), 3.14 and 3.08 (narrow AB spectrum, J = 16 Hz); CD of molybdate (VI) complex (nm, $[\Theta] \times 10^{-4}$), 279, +0.7; 250, −1.9; 233, + 0.7; 220, −0.6.

1b: $[\alpha]_D^{20}$ +11.0° (*c* 2.0, water) [lit.[4] value for the inhibitory isomer, $[\alpha]_D^{22}$ −12.4° (water)]; ^{1}H NMR (D_2O, 85 °C) δ 5.21 (d, $^2J_{HF}$ = 47 Hz), 3.11 and 2.96 (AB spectrum, J = 16 Hz); CD of molybdate(VI) complex (nm, $[\Theta] \times 10^{-4}$), 276, +1.2; 250, −2.8; 233, +1.6; 218, −0.7.

Epimerization of (1*R*,2*S*)-Trimethyl Fluorocitrate (Ester of 1a). The title ester (13 mg, GLC purity > 99%) was treated (23 °C, 3.3 h) with a solution of sodium methoxide in methanol (0.5 M, 0.5 mL), giving (GLC) approximately 50% conversion to the 1*S*,2*S* ester. After filtration through an ion-exchange column (Dowex 50W-X8, H^+ form, methanol), a small amount of diazomethane was added. The isomers were separated by using the HPLC (Partisil 10) system and were obtained pure. Hydrolysis to the acids and CD analysis of these as molybdate(VI) complexes were performed as above. Except for some minor deviations in the intensities of some peaks, the CD spectra were indistinguishable from those of **1a** and **1b**, respectively.

Reformatsky Reaction with Ethyl Bromopropionate. The two-step Reformatsky technique involving preparation of the reagent in refluxing dimethoxymethane was employed.[50] Owing to the low yield (24%) of the Reformatsky reagent obtained from ethyl bromopropionate, a large excess of this ester (36.2 g, 200 mmol) and of zinc dust (14.1 g, 220 mmol) in dimethoxymethane (120 mL) were required. When the Reformatsky reagent had been prepared (reflux 5 min), a solution of the ketone **2** (5.28 g, 20 mmol) in methylene chloride (50 mL) was added

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within 1 min to the refluxing reagent mixture. After the mixture was refluxed for an additional 5 min, workup was performed as above. GLC (3% JXR, 230 °C) showed only two large peaks (ratio ca. 1:3) having retention times (4.4 and 4.7 min, respectively) longer than that of the ketone **2** (1.3 min). On passing the solution through a silica gel (0.040–0.063 mm) column (50 × 620 mm) with methylene chloride/ethyl acetate (9:1) as solvent, most of the contaminants were removed. About 60% of each of **4a** (eluted first) and **4b** were obtained isomerically pure and a further purification on a similar column gave **4a** (2.00 g) and **4b** (4.48 g), both in a purity of about 90%; combined yield, ≈ 80%.

Methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-[(*R*)-1′-(ethoxycarbonyl)-ethyl]-α-D-*ribo*-hexopyranoside (4a) was crystallized first from cyclohexane, and then from ethanol and three times from hexane: mp, 95.5–96.5 °C; $[\alpha]_D^{25}$ +16.8° (*c* 1.0, ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (5 H), 5.53 (s, PhCH), 4.85 (H-1, broad d, $J \approx 2.9$ Hz), 4.5–3.5 (m, 7 H, including a q at 4.12, $COOCH_2$), 3.41 (s, OCH_3), 3.06 (q, $CHCH_3$), δ_A 2.03 and δ_B 2.25 (AB part of ABX spectrum, J_{AB} = 14.9 Hz, J_{AX} = 4.0 Hz, J_{BX} = 1.2 Hz), 1.25 (t, CH_3CH_2), 1.24 (d, CH_3CH); ^{13}C NMR ($CDCl_3$) 174.43 (C=O), 137.39, 128.74, 128.03, and 125.99 (aromatic carbons), 101.60 (PhCH), 98.97 (C-1), 80.38 (C-4), 71.14 (C-6), 69.25 (C-3), 60.38 ($COOCH_2$), 59.47 (C-5), 55.31 (OCH_3), 43.93 ($CHCH_3$), 34.65 (C-2), 14.30 (CH_3CH_2), 11.11 (CH_3CH).

Methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-[(*S*)-1′-(ethoxycarbonyl)-ethyl]-α-D-*ribo*-hexopyranoside (4b) was crystallized first from cyclohexane and then from ethanol: mp 114–115 °C; $[\alpha]_D^{25}$ +106° (*c* 1.0, ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (5 H), 5.54 (PhCH), 4.82 (broad d, J = 3.9 Hz, H-1), 4.5–3.45 (m, 7 H, including a q at 3.86, $COOCH_2$), 3.41 (s, OCH_3), 2.74 (q, $CHCH_3$), δ_A 2.27 and δ_B 1.82 (AB part of ABX spectrum, J_{AB} = 14.8 Hz, $J_{AX} \approx 0$ Hz, J_{BX} = 4.4 Hz), 1.18 (d, CH_3CH), 1.09 (t, CH_3CH_2); ^{13}C NMR ($CDCl_3$) 174.87 (C=O), 137.30, 128.94, 127.99, and 126.24 (aromatic carbons), 101.89 (PhCH), 98.53 (C-1), 80.82 (C-4), 71.29 (C-6), 69.20 (C-3), 60.30 ($COOCH_2$), 58.96 (C-5), 55.36 (OCH_3), 44.95 ($CHCH_3$), 36.43 (C-2), 13.87 (CH_3CH_2), 12.30 (CH_3CH).

A Reformatsky synthesis of **4a** and **4b** in THF by using activated zinc dust was carried out as for **3a** and **3b**, and the products were analyzed by capillary column GLC. Reflux of the reaction mixture of 30 min followed by hydrolysis gave 42% and 51% yields of **4a** and **4b**, respectively. Reflux for only 10 min gave a ratio of about 1:3. Five of the minor products were isolated by column chromatography and characterized by ^{1}H NMR spectroscopy. The yields and retention times below are as found by capillary column GLC: four Reformatsky products derived from **9** (1.0%, 10.77 min; 1.0%, 11.20 min; 0.7%, 11.36 min; 0.3%, 12.37 min); C-3 epimer of **4a** or **4b** (2.1%, 12.91 min); **6a** (?, not isolated, 0.3%, 16.37 min); **4a** (42.0%, 17.17 min); **4b** (51.1%, 18.24 min).

Degradation of 4a and 4b to methylcitric acids was performed as for **3a** and **3b**. GLC quantitation of the resulting trimethyl methylcitrates (comparing response with that of a standard solution) indicated a 23% yield of (2*R*,3*R*)-methylcitric acid[1] (**5a**) and a 27% yield of the 2*R*,3*S* isomer **5b**. Preparative HPLC as described above gave analytical samples. For **5b** all material was purified, giving the trimethyl ester in 28% yield, thereby confirming the GLC yield.

Trimethyl ester of (2*R*,3*R*)-methylcitric acid[1] (**5a**) was obtained from **4a** as a colorless liquid: $[\alpha]_D^{20}$ −13° (*c* 0.5, methanol); ^{1}H NMR and MS were as previously described.[31]

Trimethyl ester of (2*R*,3*S*)-methylcitric acid[1] (**5b**), obtained from **4b**, was crystallized from ether/cyclohexane; mp 26.5–28 °C; $[\alpha]_D^{20}$ +21.5° (*c* 1.2, methanol); ^{1}H NMR and MS were as previously described.[31]

Hydrolysis to methylcitric acids was accomplished as for the fluorocitric acids except that longer hydrolysis times were used (8 days).

5a: $[\alpha]_D^{20}$ +13° (*c* 0.4, water); CD as molybdate(VI) complex (nm, $[\Theta] \times 10^{-4}$), 278, +0.9; 251, −1.4; 235, +1.4 (cf. ref 31).

5b: $[\alpha]_D^{20}$ + 20.0° (*c* 1.0, water); CD as molybdate(VI) complex (nm, $[\Theta] \times 10^{-4}$), 278, +0.5; 251, −1.2; 233, +0.5 (cf. ref 31).

Reaction of 2 with cyanomethane was carried out as described.[20] The only product detected (GLC, TLC) showed mp 171–172 °C; $[\alpha]_D^{20}$ + 71° (*c* 1.0, chloroform) [lit.[20] values: mp 170 °C; $[\alpha]_D^{22}$ +69° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) spectrum was indistinguishable from that published. The same product was also obtained from **2**, cyanomethane, and lithium diethylamide in THF.

Transformation of the above nitrile to the corresponding amide **methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-[(aminocarbonyl)methyl]-α-*D*-*ribo*-hexopyranoside (7a)** was accomplished by dissolving the nitrile (0.50 g, 1.6 mmol) in ethanol (10 mL, 78 °C), removing the heating bath, and adding aqueous NaOH (1 M, 0.3 mL) and hydrogen peroxide[51] (5.7 mmol, as 35% solution). After 40 min the mixture was poured into water and the product was extracted twice with methylene chloride. Washing the combined organic layers with water, drying (Na_2SO_4), filtration, and evaporation of the solvent gave a white crystalline mass which was recrystallized from ethyl acetate to give the amide **7a** (0.49 g, 92%): mp 182.5–183.5 °C; $[\alpha]_D^{20}$ +102° (*c* 1.0, chloroform); IR ($CHCl_3$) 1678 cm^{-1} (s); ^{1}H NMR ($CDCl_3$) δ 7.6–7.1 (5 H), 6.51 (1 H, amide H), 5.59 (PhCH), 5.46 (1 H, amide H), 4.85 (broad d, H-1, X part of ABX spectrum, $J_{AX} \approx 0$ Hz, J_{BX} = 3.7 Hz, 4.5–3.45 (m, 5 H), 3.42 (s, OCH_3), 2.86 and 2.23 (CH_2CONH_2, AB spectrum, J = 14.4 Hz), δ_A 2.20 and δ_B 2.03 (H-2, AB part of ABX spectrum, $J_{AX} \approx 0$ Hz, J_{BX} = 3.7 Hz, J_{AB} = 14.4 Hz).

Reformatsky reaction with ethyl bromoacetate was carried out using the two-step procedure.[50] The Reformatsky reagent was prepared from the bromo ester (18.9 g, 112 mmol) in refluxing (30 min) dimethoxymethane. The ketone **2** (7.0 g, 27 mmol), dissolved in THF (110 mL), was added, and the resulting mixture was heated under reflux for 30 min. Workup as above and analysis by GLC (packed column) showed **6a** and **6b** to be present in the ratio 94:6. Evaporation of the solvent gave a partly crystalline mass. Recrystallization from ethyl acetate/2,2,4-trimethylpentane afforded the major component **6a** (7.0 g). The mother liquor was chromatographed on the Lobar columns with 2,2,4-trimethylpentane/ethyl acetate (7:2). This gave **6b** (0.37 g) and a further 0.65 g of **6a**; combined yield of **6a** and **6b**, 86%.

Methyl 4,6-*O*-Benzylidene-2-deoxy-3-*C*-[(ethoxycarbonyl)methyl]-α-D-*ribo*-hexopyranoside (6a): mp 90–90.5 °C; $[\alpha]_D^{20}$ +76° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (5H), 5.57 (PhCH), 4.81 (H-1, X part of ABX spectrum, J_{AX} = 1.3 Hz, J_{BX} = 3.7 Hz), 4.5–3.5 (m, 7 H), 3.40 (s, OCH_3), 2.67 and 2.58 (AB spectrum, CH_2COO, J = 14.6 Hz), δ_A 2.24 and δ_B 2.09 (H-2, AB part of ABX spectrum, J_{AB} = 14.8 Hz, J_{AX} = 1.3 Hz, and J_{BX} = 3.7 Hz), 1.19 (t, 3 H). Anal. $C_{18}H_{24}O_7$: C, H.

Methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-[(ethoxycarbonyl)methyl]-α-D-*arabino*-hexopyranoside (6b) was obtained as a colorless oil: $[\alpha]_D^{20}$ +64° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (5 H), 5.57 (PhCH), 4.77 (H-1, X part of ABX spectrum, J_{AX} = 1.5 Hz, J_{BX} = 3.9 Hz), 4.4–3.4 (m, 7 H), 3.34 (s, OCH_3), 3.14 and 2.73 (AB spectrum, CH_2COO, J = 16.4 Hz), δ_A 2.17 and δ_B 1.97 (H-2, AB part of ABX spectrum, J_{AB} = 14.3 Hz, J_{AX} = 1.5 Hz, and J_{BX} = 3.9 Hz), 1.14 (t, 3 H).

Reduction of 6a with Lithium Aluminum Hydride. Compound **6a** was reduced by standard procedures (ether, 35 °C, 1 h) to give the product **10**, which was recrystallized from ethyl acetate/2,2,4-trimethylpentane, (78% yield): mp 132–138 °C; $[\alpha]_D^{20}$ + 95 ° (*c* 1.0, chloroform). ^{1}H NMR and IR (KBr) spectra were indistinguishable from those of the product obtained by reduction of **3a** and **3b** with lithium triethylborohydride.

Synthesis of Amides from 6a and 6b. A hydrazide was prepared from **6a** by treatment of the ester with excess hydrazine hydrate in refluxing ethanol (3 h). Recrystallization from ethanol gave a product (91% yield) with mp 198–199 °C; $[\alpha]_D^{20}$ +104° (*c* 1.0, chloroform). A hydrazide was prepared similarly from **6b** but was not obtained crystalline. After purification on a silica gel column (chloroform/ethanol; 10:1), it showed $[\alpha]_D^{20}$ + 92° (*c* 1.0, chloroform). Both hydrazides showed ^{1}H NMR spectra which were similar to those of the corresponding amides **7a** (above) and **7b** (below).

Amide 7a was synthesized from the hydrazide obtained from **6a** by the action of potassium hexacyanoferrate(VI).[52] The crystalline product (from ethyl acetate) showed mp 183–183.5 °C (83% yield); $[\alpha]_D^{20}$ + 102° (*c* 1.0, chloroform). Its IR and ^{1}H NMR spectra were indistinguishable from those of the amide **7a** obtained from the nitrile (see above).

Amide 7b was synthesized similarly from the hydrazide obtained from **6b**. Purification on the Lobar columns afforded a white amorphous solid (77%): $[\alpha]_D^{20}$ +85° (*c* 1.0, chloroform); IR ($CHCl_3$) 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (5 H), 5.85 (broad s, 2 H, NH_2), 5.60 (PhCH), 4.77 (H-1), X part of ABX spectrum, J_{AX} = 1.2 Hz, J_{BX} = 3.9 Hz), 4.4–3.5 (m, 5 H), 3.36 (s, OCH_3), 2.82 (s,CH_2CO), δ_A 2.21 and δ_B 1.97 (H-2, AB part of ABX spectrum, J_{AB} = 14.4 Hz, J_{AX} = 1.2 Hz, and J_{BX} = 3.9 Hz).

Synthesis of (*S*)-[1,1-$^2H_2$]Citric Acid. The ketone **2** (1.0 g) was treated (50 °C, 90 h) with D_2O (25 mL, 99.75% *d*) and pyridine (0.5 mL) in THF (25 mL). After evaporation of the solvents, the residue was recrystallized from toluene: mp 172–173 °C; ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (5 H), 5.58 (PhCH), 5.13 (H-1), 4.5–3.6 (m, 4 H, indistiguishable from the corresponding part of the spectrum of the nondeuterated ketone), 3.38 (s, OCH_3).

The dideuterated ketone **8** was allowed to react with ethyl bromoacetate and zinc as described above for **2**, and the major product was

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crystallized from the crude product mixture. Recrystallization as above gave a 69% yield of the ethyl ester: mp 90–90.5 °C; $[\alpha]_D^{20}$ +72° (c 1.0, chloroform); ^{1}H NMR ($CDCl_3$) 7.6–7.1 (5 H), 5.58 (PhCH), 4.81 (H-1), 4.5–3.5 (m, 7 H), 3.41 (s, OCH_3), 2.67 and 2.58 (CH_2COO, AB spectrum, J = 14.6 Hz), 1.20 (t, 3 H).

Degradation of the Reformatsky product with permanganate was carried out as described above and **trimethyl (*S*)-[1,1-$^2H_2$]citrate**, obtained by reaction of the crude acid with diazomethane, was crystallized from ether to give 90 mg (35% yield calculated on the Reformatsky product): mp 76–77 °C; $[\alpha]_D^{20}$ −0.59°, $[\alpha]_{546}^{20}$ −0.73°, $[\alpha]_{365}^{20}$ −2.18° (c 6.3, methanol) (lit.[53] mp for trimethyl citrate, 78.5–79 °C); ^{1}H NMR ($CDCl_3$) δ 4.11 (s, 1 H), 3.84 (s, 3 H), 3.69 (s, 6 H), 2.91 and 2.81 (AB spectrum, J = 15.5 Hz); mass spectrum, m/e (rel intensity) M^+ 236 not observed, 177 (16), 176 (0.65), 145 (100), 144 (15), 103 (37), 101 (39), 59 (31). The abundance of the m/e 176 peak shows that a maximum of 3.5% monodeuterated ester is present.

(*S*)-[1,1-$^2H_2$]Citric acid was prepared from the trimethyl ester by hydrolysis with excess (60 mol equiv) aqueous sodium hydroxide (0.5 M, 24 h). Workup as described above for the acids yielded the product; $[\alpha]_D^{20}$ −0.96°, $[\alpha]_{546}^{20}$ −1.13°, $[\alpha]_{436}^{20}$ −1.94° (c 5.2, water) [lit.[54] value for the enantiomer, $[\alpha]_{546}^{20}$ +1.03° (c 12.6, water)]; optical rotation in saturated solution of ammonium molybdate(VI) as described by Martius and Schorre,[54] $[\alpha]_D^{20}$ −25.9°, $[\alpha]_{546}^{20}$ −31.6°, $[\alpha]_{436}^{20}$ −62.4° (lit.[54] values $[\alpha]_{546}^{20}$ −33.6° and $[\alpha]_{546}^{20}$ +31.9°); ^{1}H NMR (D_2O) δ 3.03 and 2.87 (AB spectrum, J = 15.9 Hz).

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How Many Rings Can Share a Quaternary Atom?

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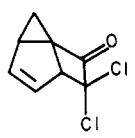
Abstract: With only primary branching, six rings may share a quaternary vertex; with secondary branching, 12 rings may be accommodated. There are two primary bicyclic systems (spiro and fused) and an additional (bridged) system when branching is considered. There are three primary tricyclic systems, termed monofuso, difuso, and trifuso (depending on the number of fusion bonds present); there are another six tricyclic systems involving secondary branching; and examples of all nine tricyclic classes are known. Without secondary branching there are two tetracyclic (trifuso and tetrafuso) and one each pentacyclic and hexacyclic classes. While the tetracyclic and higher classes with secondary branching have not been enumerated, T_d tetraadamantane is shown to possess 12 rings, the maximum possible for this class. Centropolycyclics with three-, four-, five-, and six-membered rings are enumerated and their strain energies are calculated. Although many of these polycyclic systems are known, some unknown ones are energetically accessible and should be stable. For the five-membered ring derivatives (polyquinanes), it is suggested that a small family of polydodecahedranes, analogous to the adamantanes, would be stable. When different ring sizes are allowed, a large number of polycyclics are possible. Strain calculations suggest that the five kinds of unbridged tricyclics containing three-, four-, and five-membered rings should all be reasonably stable. The proposed scheme may find use in categorizing the voluminous literature of polycyclic natural products, including alkaloids. Thus, despite a literature statement that difusotricyclics "remain rare", cephalotaxine, phytotuberin, and the cytochalasins fit this category.

Dichlorotricyclo[5.1.0.0^{1,3}]octenone (**1**), despite the ring strain



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inherent in this highly interconnected structure, proved to be surprisingly stable.[1] As we attempted to compare **1** with other possible tricyclooctanes, it became apparent that no convenient scheme for categorizing such polycyclic compounds existed. Thus, while the IUPAC rules[2a,b] and a recent graph-based nomenclature[2c] allow even the most complex ring system to be systematically named, it is usually necessary to sketch the skeleton to comprehend a given name; and it is not easy to find a given, say tricyclic, substructure buried in a higher cyclic structure. Certain groups of polycycles have been named and systematized[3] (e.g., adamantanes, polyquinanes, propellanes, fenestranes) but these are not extendable to other classes.

In considering how such molecules are assembled, it occurred to us to enumerate the ways that rings can share a common atom. This approach led to a novel way of categorizing such systems and uncovered some interesting and energetically accessible structures that have not yet been reported. Furthermore, we performed empirical force-field calculations and confirmed the potential stability of many of these systems.

How Many Rings Can Share a Vertex?

Consider a carbon atom with four valences directed toward the corners of a tetrahedron. How many rings, all containing that carbon atom, can be constructed? In order to make this problem

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