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The Absolute Configuration of (+)-3-Fluorolactic Acid*

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ABSTRACT: 3-Fluorolactic acid has been resolved. Enzyme-inhibitory activity is found to reside exclusively in the (+) isomer, and arguments are presented for the assignment of the L configuration to this enantiomer.

Mono- and difluorocarboxylic acids have proved to be valuable tools both as enzyme reagents in complex biological systems and in the study of enzymatic reaction mechanisms (Kun *et al.*, 1964; references cited in Gottwald and Kun, 1965). 3-Fluorolactic acid, required for *in vitro* enzyme kinetic experiments, was recently synthesized (Gottwald and Kun, 1965), and we now report its resolution into optical antipodes and a correlation between their absolute configuration and biochemical activity. Enzyme-inhibitory activity was found to reside exclusively in the (+) isomer, and a detailed account of the enzyme-kinetic mechanism of action on crystalline lactate dehydrogenase will appear elsewhere (Ayling and Kun, 1965).

Resolution of racemic 3-fluorolactic acid was achieved by mixing the acid with morphine in a 2:1 molar ratio by an adaptation of the method of Wood *et al.* (1926), after which morphine (−)-3-fluorolactate crystallized as the less soluble salt. This was converted into the free acid, which was in turn characterized as the sodium, zinc, and (+)-α-phenylethylamine salts. The mother liquors of the morphine salt afforded the enantiomeric (+)-3-fluorolactic acid, characterized as the sodium, barium, calcium, and (−)-α-phenylethylamine salts.

It has recently become possible (Craig and Roy, 1965) to assign the absolute configuration of α-hydroxy acids directly from a knowledge of the optical rotatory dispersion curve of the acid. Application of this method to lactic acid (Craig and Roy, 1965) gave the curves shown in Figure 1, from which the (known) D and L configurations of the two enantiomers could readily be assigned with reference to D-glyceric acid, and hence to D-glyceraldehyde (Craig and Roy, 1965). Extension of the same method to the resolved 3-fluorolactic acids (Figure 2) revealed a very close correspondence between the curves of the two (+) acids. As in previous cases (Craig and Roy, 1965), the curves of both enantiomers were recorded to ensure complete validity for the conclusions reached, in view of the risks which may be caused by scattered light or instrumental errors in the 200–220 mμ region. The curves for the two (−) acids are seen to be exact mirror images of their respective enantiomers. Similar close correspondence is revealed (Figures 1 and 2) between the rotatory dispersion curves of the anions of the two acids, and on the basis of this correspondence it is possible to assign the L configuration to (+)-3-fluorolactic acid.

This conclusion is supported by the following confirmatory evidence. Morphine forms the less soluble salt with (−)-3-fluorolactic acid and also with D-(−)-lactic acid (Wood *et al.*, 1926) and D-(−)-3-bromolactic acid (Freudenberg, 1914). In view of the close similarity of the three acids, it is reasonable to assume that the less soluble morphine salts are probably configurationally identical and that (−)-3-fluorolactic acid has the D configuration.

Second, the inhibitory isomer of fluorolactate is the (+) form, corresponding to the physiological substrate

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TABLE I: Rotations[a] of Acids of Structure I and of Their Anions.

R	H	F	Cl	Br	OH
Acid	+2.6 (8)[b]	+2.76 (12.4)	+2.60 (15)	+2.01 (10)	+3.0 (1.1)
Sodium salt	−13.7 (4)[b]	−8.35 (12.5)			−16.13 (10)
Zinc salt	−8.4° (4)	−8.0 (5)			−22.18 (10)
Barium salt		−4.3 (15)			−10.6 (6.4)
Calcium salt	−4.5 (10)	−4.8 (12.3)			−12.0 (7.6)

[a] Measured in degrees as $[\alpha]_D$ for an aqueous solution except where stated; concentrations in parentheses. [b] Measured at 546 mμ.

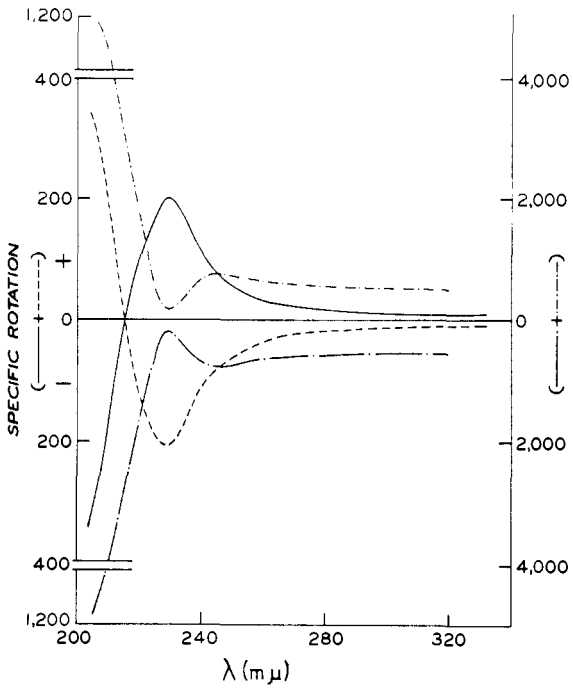


FIGURE 1: Rotatory dispersion curves of L-(+)-lactic acid (———), L-calcium lactate (——·), D-(−)-lactic acid (— —), and D-calcium lactate (— · —).

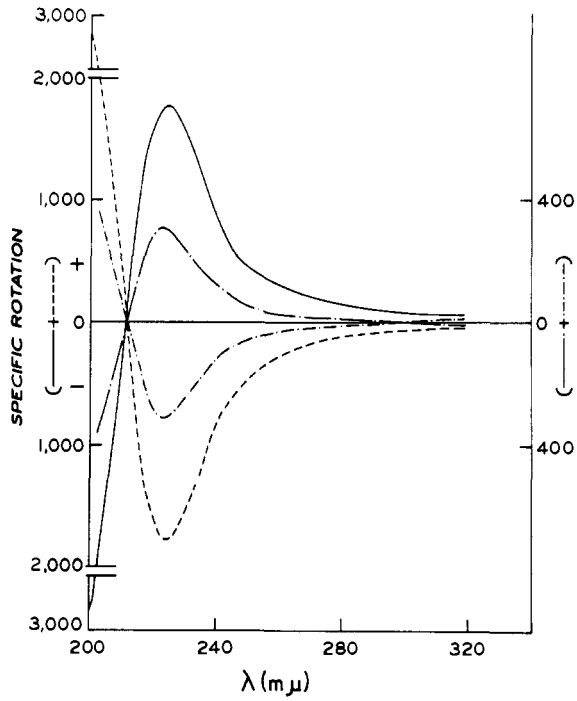
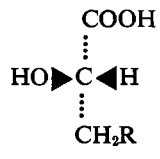


FIGURE 2: Rotatory dispersion curves of L-(+)-3-fluorolactic acid (———), L-sodium 3-fluorolactate (——·), D-(−)-3-fluorolactic acid (— —), and D-sodium 3-fluorolactate (— · —).

of lactate dehydrogenase, *i.e.*, L-(+)-lactic acid.[1] Since an enzyme is the most sensitive stereospecific reagent known, the configurational identity of the two active enantiomers receives strong support from this observation. In this connection it is interesting that malic dehydrogenase (Davies and Kun, 1957) oxidizes only the unnatural and *meso* forms of tartaric acid; the former has the L-(−) configuration (Craig and Roy, 1965).

Third, Table I lists the rotations at the D line for several acids of structure I (where R = H, F, Cl, Br, and OH) and (where data is available) for their anions, all of the L configuration and of appreciable and uniformly negative rotation. The closely related (+)-3-chloro- (Tsunoo, 1935) and (+)-3-bromolactic acids (Freudenberg, 1914) have been shown to possess the L configuration by reduction to L-(+)-lactic acid. From the data in Table I also, (+)-3-fluorolactic acid behaves as an L acid.



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[1] Lactic dehydrogenase recognizes (+)-3-fluorolactate in exactly the same way as it does the substrate (+)-lactate, as expressed by inhibition kinetics of purely competitive type.

In conclusion, arguments have been presented for the assignment of the L configuration to (+)-3-fluorolactic acid on the basis of (1) the close correspondence in the optical rotatory dispersion data between L-(+)-lactic and (+)-3-fluorolactic acid and their anions, (2) the lower solubility of the morphine salts of the D-(−) acid in this and in two other cases, (3) the close similarity of the rotations of the several L acids and anions of structure I, and (4) the biochemical equivalence of L-(+)-lactic and (+)-3-fluorolactic acids, implying a close correspondence in their three-dimensional molecular architecture.

Experimental Section

(−)-3-Fluorolactic acid was prepared by resolution of the racemic acid using morphine. (±)-3-Fluorolactic acid (26.5 g, 245 mmoles) and 37.9 g (125 mmoles) of morphine monohydrate were dissolved in water and allowed to crystallize. After four fractional crystallizations, 27.3 g (93 mmoles, 76% yield) of morphine (−)-3-fluorolactate was collected, mp 222–223° dec, $[\alpha]_D^{25}$ −90.8° (*c* 5, water). The morphine was regenerated with ammonium hydroxide, and the free acid was obtained by ion exchange on a Dowex 50 column, giving 50 ml of a final solution containing 8.59 g (79.5 mmoles, 65%) of (−)-3-fluorolactic acid, $[\alpha]_D^{25}$ −2.74° (*c* 17.18, water). The sodium salt had $[\alpha]_D^{25}$ +8.34° (*c* 10.8, water) and the zinc salt had $[\alpha]_D^{25}$ +8.00° (*c* 5, water). Neutralization of the acid with (+)-α-phenylethylamine afforded (+)-α-phenylethylammonium (−)-3-fluorolactate crystallizing from ethanol as prisms of a monohydrate; mp 87–88°, $[\alpha]_D^{25}$ +7.30° (*c* 5.0, water).

Anal. Calcd for $C_{11}H_{16}FNO_3 \cdot H_2O$: C, 53.43; H, 7.34; N, 5.66. Found: C, 53.49; H, 7.35; N, 5.67.

(+)-3-Fluorolactic acid was obtained from the mother liquors of the morphine (−)-3-fluorolactate. The solution was treated with ammonium hydroxide, and the free acid was obtained by ion exchange using a Dowex 50 column. Neutralization of the acid with (−)-α-phenylethylamine gave, after four fractional crystallizations from ethanol, 8.20 g (35.8 mmoles, 29%) of (−)-α-phenylethylammonium (+)-3-fluorolactate as prisms of the monohydrate, mp 87–88°, $[\alpha]_D^{25}$ −7.30° (*c* 5.0, water).

Anal. Calcd for $C_{11}H_{16}FNO_3 \cdot H_2O$: C, 53.43; H, 7.34; N, 5.66. Found: C, 53.29; H, 7.20; N, 6.04.

Treatment with sodium hydroxide solution, extraction of the amine with benzene, and recovery of the free acid by ion exchange gave 3.11 g (28.8 mmoles, 24%) of (+)-3-fluorolactic acid, $[\alpha]_D^{25}$ +2.76° (*c* 12.44, water). The following salts were prepared: sodium salt, $[\alpha]_D$ −8.35° (*c* 12.57, water); barium salt, $[\alpha]_D$ −4.3° (*c* 15.0, water); and calcium salt, $[\alpha]_D$ −4.8° (*c* 12.3, water).

Rotatory Dispersion Curves. These were measured with a Bendix Model 460-C or Cary Model 60 spectropolarimeter using 1-mm or 1-cm cells at 25° and were reproducible to within 5%. Rotations are given below only for (1) the highest and lowest wavelengths measured and (2) peaks and troughs. Since measurements taken for enantiomeric pairs agreed within 5%, the dispersion curve of only one isomer is described; *(+)-3-fluorolactic acid*: RD (*c* 2.16, water): $[\alpha]_{320}$ +55.5°, $[\alpha]_{224.5}$ +1778° (peak), $[\alpha]_{200}$ −2732°; *(−)-sodium 3-fluorolactate* (from (+)-3-fluorolactic acid): RD (*c* 1.4, water): $[\alpha]_{320}$ −10.73°, $[\alpha]_{222.5}$ −307.5° (peak), $[\alpha]_{203}$ −357.5°; L-(−)-*calcium lactate*, $[\alpha]_D$ −4.5° (*c* 10.0, water): RD (*c* 0.61, water): $[\alpha]_{320}$ −52.3°, $[\alpha]_{240}$ −73.6° (trough), $[\alpha]_{225}$ −65.4° (peak), $[\alpha]_{205}$ −1112°.

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