ENZYMATIC INVERSION AT SATURATED CARBON: NATURE AND MECHANISM OF THE INVERSION OF R(-) p-iso-BUTYL HYDRATROPIC ACID*

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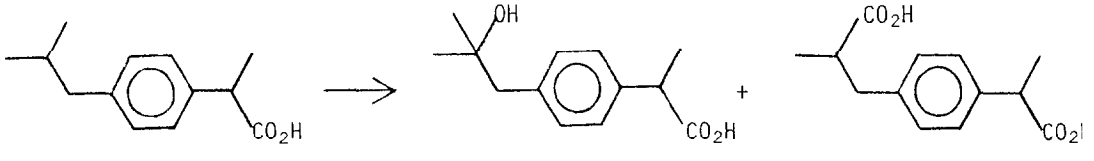
Summary. We propose the existence of a previously unrecognized enzymatic pathway in man which allows for optical inversion (epimerization) at saturated carbon, employing R(-)-p-iso-butyl hydratropic acid (I) and an analog in which the chiral center and methyl group were deuterium labeled [R(-)d_4]. We have proposed a detailed enzymatic pathway for this optical inversion in which we postulate the existence of an R-aryl propionic acid isomerase system. The results make understandable the bioequivalence of a variety of (S) and (R) isomers of non-steroidal anti-inflammatory agents.

Introduction. Our curiosity concerning the metabolism of the non-steroidal anti-inflammatory agent d,l p-iso-butyl hydratropic acid (I, ibuprofen, Motrin®) was elicited by the report of Adams et al. (1) that the urinary metabolites II and III were optically active and further that the individual S(+) and R(-) isomers of this drug were essentially biologically equivalent in vivo (2). Since many of the anti-inflammatory hydratropic acids do not exhibit this latter behavior (only the S isomer is biologically active) we felt that it was important to understand the basis for this apparently anomalous behavior. Consequently, we designed a series



I II III

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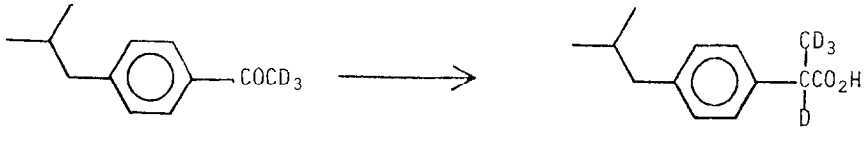
of experiments in man to determine the stereochemical course of the metabolism of Compound I and ultimately the relative stereochemical relationships between the optical antipodes of I and the products of mammalian metabolism.

Materials and Methods. Initially, we resolved the acid (I) into its optical isomers and administered sequentially 800 mg doses (with one-week spacing between experiments) of the S(+), R(-) and racemic I to three human volunteers. These individuals collected their urine during the next 72 hours. (0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60 and 60-72 hours samples were pooled). In addition, the plasma levels of the optical antipodes were monitored by the method of Kaiser and VanGiessen (3) employing GLC of their diastereomeric amides. Mass spectroscopic evaluation of the deuterium labeled I [R(-)d_4] was used to identify metabolic removal of deuterium. The deuterium labeled analog of I, Compound V was synthesized by the method of Wu and White (4) from the conveniently available trideutero-p-iso-butyl acetophenone IV. In the final hydrolysis step NaOD/D_2O was substituted for the normal aqueous alkaline hydrolysis in order to place a deuterium atom on the asymmetric methine carbon. The structure of this product was



IV V

confirmed both by mass spectroscopy (M + = 210) and its nmr spectrum, which was essentially devoid of the methyl and methine resonances characteristic of the hydratropic acid side chain owing to deuterium substitution. The tetradeutero Compound V was resolved in the same manner as its parent acid I to give R(-)d_4 (Fig. 2).

Results and Discussion

The results of the above investigation with the isomer of I are

Table I

DRUG ADMINISTERED	URINARY PRODUCTS BY GLC
S(+) I	S(+) I; S(+) II
R(-) I	S(+) I, 80%; R(-) I, 20%; S(+) II, 54%; R(-) II, 46%
RACEMIC I	S(+) I, 71%; R(-) I, 30%; S(+) II, 71%; R(-) II, 29%

summarized in Table I. They indicate that there is a facile epimerization of the R(-) isomer of I to its optical antipode S(+), I. This fact would account for the essential bioequivalence of the S(+) and R(-) isomers. These results suggest further that the metabolism is competitive with the epimerization reaction, for which only the R(-) isomer is apparently a substrate. Analysis of the configuration of the products of the R(-) isomer indicates that both optical isomers are metabolized and that the k (metabolism) to give II is roughly equivalent to k (isomerization) since the product II was about equally divided between R(-) and S(+) isomers. The more complex question of the diastereomeric metabolite III will be dealt with in future communications.

We now questioned the course of this unique reaction (5,6) in man. That the reaction was not artifactual was established by the fact that R(-) I is optically stable between pH 1 and 10 for 24 hours at 37°. It is also evident that such a stereoselective reaction could not result from any such non-discriminatory mechanism. The energy requirements of an intermediary carbonium ion, carbanion or radical mitigate against such species arising in a biological system; the stereoselectivity reinforces this supposition. We thus hypothesized the existence of an R-aryl-propionic acid isomerase (R-APAI) enzyme system proceeding via the enzymes of lipid catabolism and anabolism as outlined in Fig. 1. It is assumed that the CoA ester of R(-) I, [R], is a substrate for fatty acid dehydrogenase, thus eliminating the chiral center. The third step may or may not

EPIMERIZATION MECHANISM R-APAI

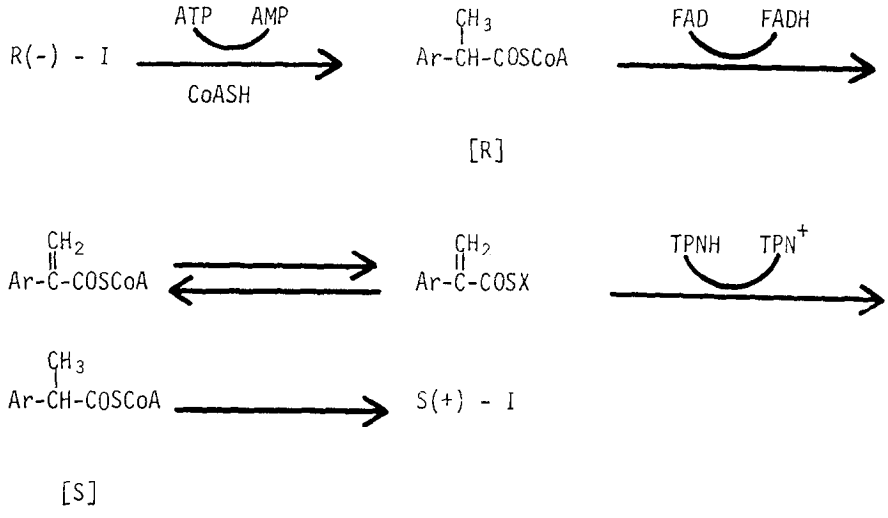
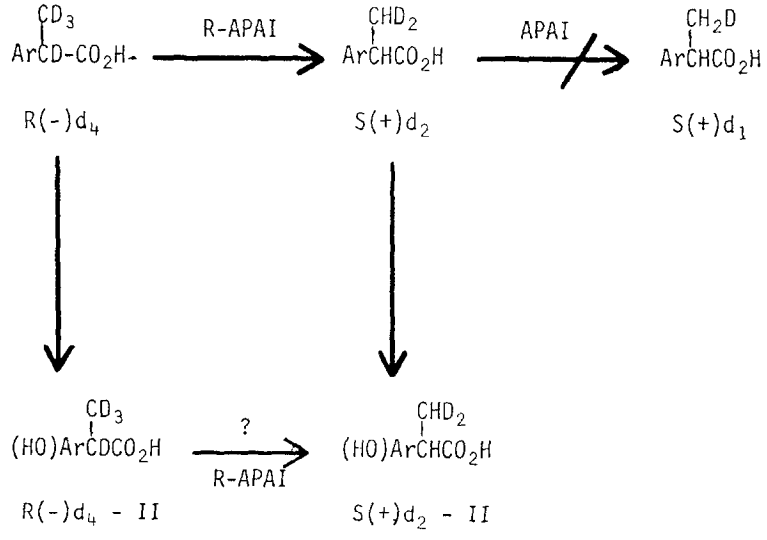


FIG. I

HUMAN METABOLISM



d R(-)/dt:d R(-)d_4/dt≈2

FIG. 2

take place depending on whether or not the CoA ester must be transferred to acyl carrier protein or another site in the fatty acid synthetase system, so that a stereoselective reduction by enoyl reductase may take place. Thus, the nature of X is unknown. The details of these enzymatic steps will be the subject of a future report on in vitro investigations.

In order to support these speculations, R(-)d_4 (Fig. 2), the resolved deuterated analog of I, was prepared and the human experiment repeated. Consistent with the mechanism outlined in Fig. 1, the metabolism followed the route described by Fig. 2. The epimerized S(+) analog of I contained predominantly two deuterium atoms, S(+)d_2. This latter substance was not a substrate for the enzyme system (no S(+)d_1 or d_0 was produced). Thus, we propose the nomenclature R-aryl propionic acid isomerase (R-APAI). The finding that all metabolite (blood and urine) II of the R configuration contained four deuterium atoms (R(-)d_4-II) while metabolite II in the S configuration contained only two deuterium atoms S(+)d_2-II gave additional credibility to this hypothesis. Comparison of the results from measurement of the rates of disappearance of R(-)d_4-I and R(-)I from plasma strongly suggests that the dehydrogenation reaction is the rate determining step in the R-APAI system.

The details of these and other metabolic studies will be the subject of future communications.

References

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