and references cited therein.
(8) G. H. Spencer, P. C. Cross, and K. B. Wiberg, *J. Chem. Phys.*, **35**, 1925 (1961).
(9) D. S. King, Ph.D. Thesis, University of Pennsylvania 1976.
(10) K. Clasius and U. Luthi, *Helv. Chim. Acta*, **40**, 445 (1957).
(11) Enriched 1,4-*s*-tetrazine-$^{15}N_2$ was prepared previously by Merer and Innes from glycine-$^{15}N_1$. The present route from sodium nitrite-$^{15}N_1$ proved considerably more economical: A. J. Merer and K. K. Innes, *Proc. R. Soc., London, Ser. A*, **302**, 271 (1969).
(12) P. W. Bridgeman, *Proc. Am. Acad.*, **60**, 305 (1925); also see D. C. Stockbarger, *J. Opt. Soc. Am.*, **39**, 731 (1949), and H. Guggenheim, *J. Appl. Phys.*, **34**, 2482 (1963).
(13) For further details on the nature of high resolution condensed phase spectra see R. M. Hochstrasser and P. N. Prasad in "Excited States", E. C. Lim, Ed., Academic Press, New York, N.Y., 1974, p 79; R. M. Hochstrasser and C. J. Marzzacco, *J. Chem. Phys.*, **49**, 971 (1968); J. P. Byrne and I. G. Ross, *Aust. J. Chem.*, **24**, 1107 (1971).
(14) The mass spectra were obtained on a DuPont 21-492 double focusing spectrometer with a V. G. Data Systems 2040 data system. The mass spectrometer was operated so that M/ΔM = 8000. This equipment, which is not modified for high accuracy isotope ratio determinations, ignored peaks with intensities less than 1.5% of the most intense peak.
(15) By process 1b we do not exclude the equivalent possibility of initial HCN extrusion.

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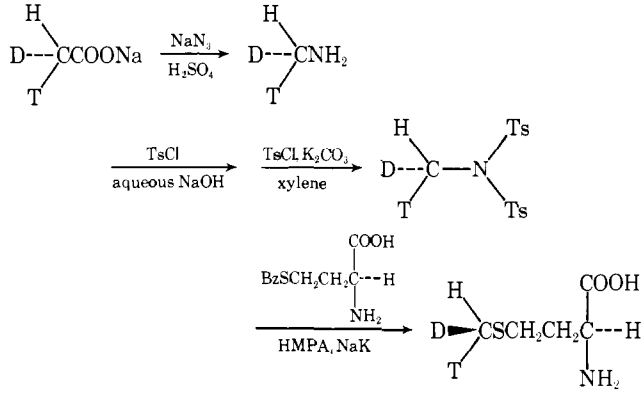
Synthesis of Methionine Carrying a Chiral Methyl Group and Its Use in Determining the Steric Course of the Enzymatic C-Methylation of Indolepyruvate during Indolmycin Biosynthesis

Sir:

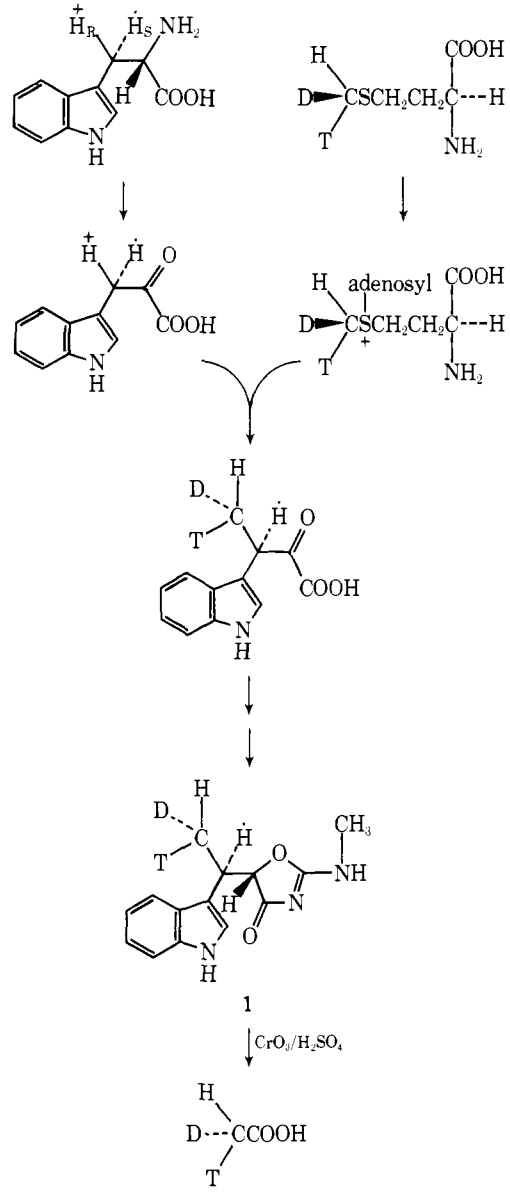
Transmethylation reactions involving the transfer of the methyl group of methionine are widespread in nature, but little is known about their detailed mechanism. This communication describes a synthesis of the two diastereomers of L-methionine carrying a chiral methyl group and the results of experiments using these substrates as methyl donors in the in vivo synthesis of the antibiotic, indolmycin (**1**).

The reaction sequence for the methionine synthesis is shown in Scheme I. The *S*- and *R*-[2-$^2H_1$,$^3H_1$]acetates needed as starting materials were prepared by using the glycolytic enzymes to synthesize *R*- and *S*-[3-$^2H_1$,$^3H_1$]pyruvates,[1,2] which were trapped as lactate using an excess of lactate dehydrogenase and NADH. Lactate was isolated by paper chromatography (Whatman 3MM, ethanol–NH_4OH–H_2O 8:4:17) and oxidized to acetate.[3] The chirality of the methyl group of these acetate samples and others described below was determined by the method of Cornforth et al.[4] and Arigoni et al.,[5] following

Scheme I



Scheme II



Eggerer's procedure.[4] In this procedure the acetate sample is enzymatically converted to malate which is then equilibrated with fumarase. During equilibration, malate samples synthesized from the *S*-isomer of acetate retain less than half of their tritium while samples synthesized from the *R*-isomer retain more than half of their tritium.

The first step in the synthesis of methionine is the Schmidt degradation of acetate to methylamine,[6] which is trapped as the hydrochloride and tosylated by heating with *p*-toluenesulfonyl chloride in 10% aqueous NaOH. The second tosyl group is introduced by refluxing the monotosylate, *p*-toluenesulfonyl chloride, and K_2CO_3 in anhydrous xylene. The key step in the sequence is the use of the ditosylimide function as a leaving group in the alkylation of the homocysteine anion with the chiral methyl group.[7,8] This reaction was performed, under an argon atmosphere, by treating a suspension of benzyl-L-homocysteine in HMPA with Na-K alloy, followed by addition of the ditosylimide and heating to 80 °C. Methionine was purified by ion exchange (Dowex 50W, 10% NH_4OH) and thin layer (silica gel 60 F-254, 1-butanol–acetic acid–H_2O 4:1:1) chromatography. The Schmidt reaction is known[9] to proceed with retention of configuration and the ditosylimide displacement should involve inversion of configuration, racemization being the only plausible alternative.[10] Thus, *S*-[2-

$^{14}C,^{2}H_1,^{3}H_1$]acetate by this route gave methionine containing a methyl group which, if chiral, must have *R* configuration and *R*-[2-$^{14}C,^{2}H_1,^{3}H_1$]acetate gave (*methyl-S*)-[*methyl*-$^{14}C,^{2}H_1,^{3}H_1$]methionine. The overall yields in these syntheses were about 10% and the T/^{14}C ratios of starting material (e.g., 7.01 and 9.91) and product (e.g., 7.04 and 9.43) were essentially unchanged.

Previous work from this laboratory[12] has shown that *Streptomyces griseus* (ATCC 12648) incorporates the methyl group of methionine into the *C*-methyl and *N*-methyl groups of the antibiotic, indolmycin. This system was used to probe the steric course of a C-methylation reaction, the transfer of the methyl group of adenosylmethionine to the methylene carbon of indole 3-pyruvate.[13] Employing procedures described previously,[12] we incubated (*methyl-R*)- and (*methyl-S*)-[*methyl*-$^{14}C,^{2}H_1,^{3}H_1$]methionine (T/^{14}C 7.04 and 7.08, respectively) with shake cultures of *S. griseus* and isolated the resulting indolmycin (T/^{14}C 7.12 and 6.94). Following removal of the *N*-methyl group by alkaline hydrolysis, the indolmycin was further degraded by Kuhn–Roth oxidation to give acetic acid from the *C*-methyl group[12] (T/^{14}C 6.47 and 6.99, respectively). The chirality of the two specimens of acetate was determined as indicated above (39 and 63% tritium retention in the fumarase reaction, respectively) and the results were compared to those obtained in the chirality analysis of the original acetate samples used for the synthesis of the methionines (31 and 71% tritium retention in the fumarase reaction, respectively).

These results show that the above synthesis of methionine did produce material containing a chiral methyl group. The acetate obtained from the degradation of indolmycin had the same configuration as the acetate used for the synthesis of the respective methionine. Since the synthesis of methionine from acetate involves one inversion of configuration, it follows that the C-methylation of indole 3-pyruvate proceeds with net inversion of configuration at the methyl group.[14] This result complements our earlier finding[15] of retention of configuration at the methylene carbon of indole 3-pyruvate in this reaction and allows us to describe the stereochemistry of the process as shown in Scheme II. The somewhat (40%) lower stereochemical purity of the methyl group in the product compared to the starting acetic acid would indicate that some racemization takes place during one of the chemical steps, e.g., the alkylation of homocysteine or the Kuhn–Roth oxidation or, less likely, that the enzymatic methylation reaction is not entirely stereospecific.

Methyl transferase reactions are believed to proceed by nucleophilic attack on the methyl group of *S*-adenosylmethionine[16] and Hegazi et al.supc17 have concluded on the basis of secondary isotope effect measurements that catechol-*O*-methyl transferase involves an S_N2-like transition state. Inversion of configuration at the methyl group, as observed in this study, indicates that an odd number of nucleophilic displacements takes place and thus suggests that the methyl group is transferred directly from the donor to the acceptor without generation of a methylated-enzyme intermediate.

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References and Notes

(1) I. A. Rose, *J. Biol. Chem.*, **245**, 6052 (1970).
(2) H. G. Floss, D. K. Onderka, and M. Caroll, *J. Biol. Chem.*, **247**, 736 (1972).
(3) H. Simon and H. G. Floss, "Bestimmung der Isopenverteilung in markierten Verbindungen", Springer-Verlag, Berlin, 1967, p 50.
(4) J. W. Cornforth, J. W. Redmond, H. Eggerer, W. Buchel, and C. Gutschow, *Eur. J. Biochem.*, **14** (1970).
(5) J. Lüthy, J. Retey, and D. Arigoni, *Nature (London)*, **221**, 1213 (1969).
(6) H. Simon and H. G. Floss, ref 3, p 26.
(7) P. J. DeChristopher, J. P. Adamek, G. D. Lyon, J. J. Galante, H. E. Haffner, R. J. Boggio, and R. J. Baumgarten, *J. Am. Chem. Soc.*, **91**, 2384 (1969).
(8) N. H. Andersen and H.-s. Uh, *Synth. Commun.*, **2**, 292 (1972).
(9) E. L. Eliel, "Stereochemistry of Carbon Compounds", McGraw-Hill, New York, N.Y., 1962, p 119.
(10) Arigoni and co-workers have independently developed a similar method for the synthesis of chirally labeled methionine and have determined that this displacement does indeed occur with inversion of configuration.[11]
(11) D. Arigoni, paper presented at the International Symposium on Stereochemistry, Kingston, Canada, June 27–July 2, 1976.
(12) U. Hornemann, L. H. Hurley, M. K. Speedie, and H. G. Floss, *J. Am. Chem. Soc.*, **93**, 3028 (1971).
(13) M. K. Speedie, U. Hornemann, and H. G. Floss, *J. Biol. Chem.*, **250**, 7819 (1975).
(14) Arigoni and co-workers have fed chiral methionine to *P. shermanii* and found that the incorporation of chiral methyl groups into the corrin system of vitamin B_{12} also occurred with inversion of configuration.[11]
(15) L. Zee, U. Hornemann, and H. G. Floss, *Biochem. Physiol. Pflanz.*, **168**, 19 (1975).
(16) S. H. Mudd and G. L. Cantoni, "Comprehensive Biochemistry" Vol. 15, M. Florkin and E. H. Stotz, Ed., Elsevier, Amsterdam, 1964, p 28.
(17) M. F. Hegazi, R. T Borchardt, and R. L. Schowen, *J. Am. Chem. Soc.* **98**, 3048 (1976).

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Application of a Novel Thermistor Mercury Electrode to the Study of Changes of Activity of an Adsorbed Enzyme on Electrochemical Reduction and Oxidation

Sir:

The strong adsorption of the amino acid cystine and proteins (e.g., albumin and insulin) from aqueous solutions onto a mercury electrode surface, presumably through interaction of the disulfide bond with mercury, has been reported.[1-3] The question of whether enzymes adsorbed on mercury maintain their activity, how this activity is changed upon electroreduction, and the application of electrodes based on such adsorption for analytical purposes is the subject of this communication. It is known that enzymes covalently bound to surfaces or immobilized in gels can be employed as catalysts,[4,5] but that metal ions, such as Hg^{2+}, often deactivate enzymes. The activity of the adsorbed enzyme was determined in this study by measuring temperature changes resulting from the action of the enzyme urease on a substrate (urea) solution; similar techniques have previously been employed in studies involving dissolved enzymes.[6]

The thermistor mercury electrode (tme) was constructed by sealing a Veeco Engineering Co. 32A223 thermistor in a U-shaped glass tube with epoxy cement. A small drop of mercury (0.1 ml) covers the thermistor and serves as both the adsorption surface for the enzyme and to conduct heat from the electrode surface to the thermistor. Electrical contact to the mercury is made with a fine Pt wire. Electrodes containing thermistors in close proximity to solid metals have previously been described for measurement of heats of electrode reactions.[7] Temperature changes, determined using a dc Wheatstone bridge arrangement, followed previous practice.[6] The thermostat bath at 25 °C was maintained constant to within 0.001 °C.

When urease is adsorbed on a tme by immersion for several minutes in a solution of 4×10^{-5} M urease, then washed and immersed in a pH 7.3 phosphate or Tris buffer alone and subjected to cyclic voltammetry, the characteristic reduction and oxidation peaks of an adsorbed material (similar to those found with cystine, insulin, and albumin[1-3]) are observed (Figure 1a). The peak currents are directly proportional to scan rate, v; Epc = −0.58 V vs. sce and Epa = −0.52 V vs. sce. The