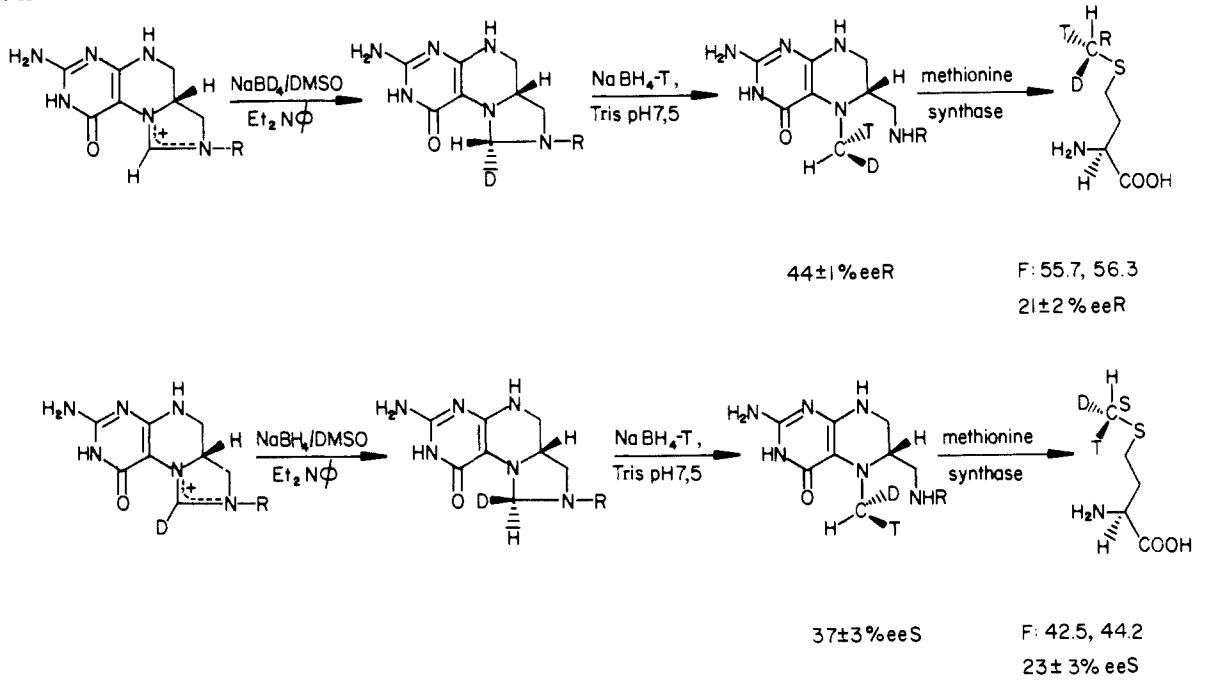
Scheme III



the methyl group (Scheme I). F values[9] of 37.2 and 37.5 (duplicate analyses) for the acetate from the first sample and 59.8 and 61.4 from the second indicated predominant S and R configuration, respectively, of the two acetate specimens. Since the degradation sequence involves one inversion of configuration in the cyanide displacement step to give acetonitrile, it follows that the first and the second 5-CH_3-H_4folate sample contained 44 ± 1% ee R and 37 ± 3% ee S isomer, respectively (Scheme III). Hence the reduction of 5,10-CH_2-H_4folate had occurred stereoselectively, exhibiting a 2–3-fold preference for attack syn to C_6-H of the 5,10-CH_2-H_4folate molecule.

Samples of the chiral methyl-R and methyl-S 5-CH_3-H_4folate were then incubated with purified cobalamin-dependent methionine synthase from *E. coli*[10] in 0.1 M potassium phosphate buffer, pH 7.2, in the presence of 25 mM dithiothreitol, 50 μM aquocobalamin, 0.5 mM homocysteine, and 19 μM S-adenosylmethionine to give methionine in about 80% yield. Methionine was separated from residual 5-CH_3-H_4folate by passage over a column of AG1X8 (Bio-Rad) and then purified by HPLC on an ODS column equilibrated with 0.1 M ammonium acetate, pH 3.55. Methionine eluted at 5–7 min,[11] and residual ammonium acetate was removed by lyophilization. The purified methionine samples were degraded by the procedure of Arigoni and coworkers[12,13] (Scheme II) to recover the methyl group as acetate for chirality analysis. This degradation sequence involves two inversions of the methyl group configuration, one in the displacement of methyl from S-methylmethionine by p-nitrothiobenzoate anion and another in the reaction of methyl p-nitrothiobenzoate with cyanide to give acetonitrile. Thus, the configuration of the acetate from the degradation directly reflects that of the methionine methyl group. F values of 42.5 and 44.2 (duplicate experiments) for the material derived from methyl-S-5-CH_3-H_4folate and 56.3 and 55.7 for that from methyl-R-5-CH_3-H_4folate indicated that the product of the enzyme reaction contained methyl groups of predominantly the same configuration as the substrate (Scheme III).

The results demonstrate that the cobalamin-dependent methionine synthase from *E. coli* transfers the methyl group of 5-CH_3-H_4folate stereoselectively to the sulfur of homocysteine to generate methionine with net retention of configuration. The observed steric course is consistent with the postulated mechanism of the reaction, which invokes two sequential transfers of the methyl group, one from 5-CH_3-H_4folate to cobalt to generate enzyme-bound methylcobalamin and a second from cobalt to sulfur to produce methionine. There is a substantial decrease in the chiral purity of the methyl group during the overall process. Since the degradation procedures for both CH_3-H_4folate and methionine are highly stereospecific,[4,5,12,13] it follows that the enzymatic methyl transfer is accompanied by nearly 50% racemization. This observation may provide a clue for the further mechanistic analysis of the reaction catalyzed by cobalamin-dependent methionine synthase.

Acknowledgment. We thank Kyungok Lee for the careful execution of the chirality analyses of acetate and the National Institutes of Health (Grants GM 32333 to H.G.F., GM 24908 to R.G.M, and GM 24729 to S.J.B. and postdoctoral fellowships ES 05306 to T.M.Z. and GM 08522 to L.F.C.) for financial support.

(10) Frasca, V.; Matthews, R. G., unpublished method. The specific activity of the purified enzyme preparations used for these experiments was 0.86–3.72 IU (μmol min^{-1} mg^{-1}), and the enzyme was about 25–80% pure as assessed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

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β-Eliminations in Isonitriles

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Received January 6, 1986

The physical organic chemistry of the nitrile group has been the subject of intensive investigations[1] which have shown that is

(1) "The Chemistry of the Cyano Group"; Rappoport, Z., Ed.; Wiley: New York, 1970.

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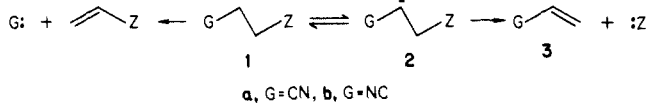
Table I. Elimination in Isonitriles, G-CH_2CH_2-Z[a]

substrate	$k(^-CN)$[b]	$k(Z^-)$[b]	activation ratio[c] -CN/-NC	products (%) from isonitriles: ^-CN	Z	CH_2=CH-Z	CH_2=CH-NC
4a, CN-CH_2CH_2-OTs		1.3×10^{-1} [r]	3.8×10^4		82[d]		64[e]
4b, CN-CH_2CH_2-SO_2Ph	4.1×10^{-4}	1.1×10^{-4} [h]	1.6×10^4	79[f]	65[g]	15	7[e]
4c, CN-CH_2CH_2-SPh	3.1×10^{-5} [k]	3.2×10^{-6} [h,k]	3.1×10^3	13[f]	10	82[h]	52[i]
4d, CN-CH_2CH_2-OPh	6.9×10^{-5} [l]	2.2×10^{-6} [h,l]	4.3×10^3	27[f]	25	74[h]	72[j]
4e, CN-CH_2CH_2-CN	3.0×10^{-2} [s]		*p*	*m*	88[n]		
4g, CN-CH_2CH_2-Ph				*q*			

[a] Reactions in EtONa-EtOH at 25 °C unless otherwise stated. [b] $dm^3\ mol^{-1}\ s^{-1}$. [c] Data for nitriles from refs. 3 and 7. [d] As NaOTs. [e] Trapped as **4c** with PhS^--EtOH. [f] By cyanide-sensitive electrode. [g] As $PhSO_2CH_2CH_2OEt$. [h] By UV spectroscopy. [i] By trapping as **4e** with KCN under phase-transfer conditions. [j] By HPLC using product from **4a** to calibrate and assuming 100% conversion. [k] Extrapolated from data from reactions >40 °C. [l] Extrapolated from data from reactions >45 °C. [m] Detected but not accurately determined. [n] As NC-CH_2CH_2-OEt. [p] Substantial for KCN-Me_2SO reactions, see text. [q] No $\bar{C}N$ after 18 h at 100 °C in M-EtONa-EtOH. [r] Reactions followed by pH stat. [s] Followed by appearance of NC-CH_2CH_2-OEt on GLC.

Scheme I



a, G=CN, b, G=NC

is a moderate carbanion stabilizer ($pK_a^{Me_2SO}$ CH_3CN 31.3, $MeSO_2Me$ 31.1, MeCOMe 26.5)[2] and hence activates elimination reactions which involve expulsion of β-substituents (Scheme I).

Quantitative data[3] for a large number of cyano-activated substrates (**1a**), with leaving groups Z = halogen, 'onium, SO_2Ph, SPh, OPh, etc.) have been obtained, and concerted or stepwise mechanisms were assigned on the basis of detritiation rates, calculated deprotonation rates, or exchange experiments.[4] It was notable, that the nitrile group was itself never expelled in such eliminations in protic solvents.[5] Because of the extraordinarily low nucleofugality[6] of the cyano group, we have been prompted to examine the *isonitrile* group as both activator and nucleofuge in 1,2-eliminations.

Activation of elimination by the isonitrile group is compared (Table I) with that for the nitrile group and it can be seen that for all leaving groups, the nitrile group is a substantially superior activator of elimination. The mechanism for elimination of the nucleofuge, Z, is the same in both series; exchange of protium for deuterium adjacent to both functions in EtOD solutions is faster for substrates **4b–d** than elimination, and we assign the $(E_1cB)_R$ mechanism for these eliminations. For tosylate **4a**, no such exchange can be detected, and for this substrate either the E_2 or the $(E_1cB)_I$ mechanism operates. For the isomeric nitrile the $(E_1cB)_I$ mechanism has been postulated.[7] These results are all in accord with qualitative observations on isonitriles which suggest rather lower acidities than for nitriles.[8]

There are considerable differences in the nitrile/isonitrile activation ratios; as the leaving group becomes less inductive in the progression SO_2Ph > OPh > SPh[9] the ratio decreases. We attribute this trend to a lower sensitivity to induction of the pre-equilibrium formation of the carbanion in the isonitriles than in the nitriles. We are currently engaged in detritiation experiments to test this possibility.

The other striking feature of Table I is the elimination of the isonitrile group under activation by the group Z. Elimination of CN^- from isonitriles, e.g., from *m*-$NO_2C_6H_4CH_2C(NC)Ph_2$, by the action of 50% $NaOH/C_6H_6/n$-$Bu_4N^+Br^-$ has been disclosed previously[10] but no rate data were reported.

For sulfone **4b** exchange experiments (EtOD) confirm that the $(E_1cB)_R$ mechanism operates for the expulsion of both cyanide and sulfinate from this substrate. The calculated deprotonation rate constant together with the rate constant for the elimination of cyanide ion allows calculation of the rank (≡nucleofugality = $\log k_{obsd} - \log k_{deprotonation} + 11$)[6] of the isonitrile group. Its rank of 3.3 places it well below leaving groups where C–halogen, C–S, C–O, or other C–N bonds are broken ($NMeAc^-$ 6.6, NMe_2Ph 10.7).[3] This value should obviously be compared with that of cyanide. Unfortunately, a direct comparison is impossible with the *nitrile* isomer of **4b**; the sole of elimination product is benzenesulfinate ion.[3] The rank (5.8) for isonitrile obtained from the nitrile–isonitrile **4e** is not less than 10 units greater than for cyano in the same system.[3]

The failure to observe loss of cyanide ion from tosylate **4a** is not surprising; the rapid elimination of tosylate ion must preclude the alternative pathway. Much more surprising is the loss of cyanide from the sulfide **4c** and even more so from the phenyl ether **4d**. We have established that for both substrates hydrogen–deuterium exchange with perdeuterioethanol as solvent is faster than elimination of either leaving group in both substrates. The isonitrile group is powerfully inductive ($\sigma_I = 0.63$),[11] and this may account for deprotonation adjacent to the phenoxy group. Another remarkable example of a phenoxy-activated elimination has recently been reported[12] to occur in a platinum complex.

Isonitrile **4g** is stable to ethoxide–ethanol, and exchange occurs slowly adjacent to, and only adjacent to, the isonitrile group.

Behavior of the three possible nitrile–isonitrile isomers **4e**, **4f**, and **5** toward $K^{13}CN$ in Me_2SO-d_6 shown by ^1H and ^{13}C NMR

CN-CH_2CH_2-NC **4f** NC-CH_2CH_2-CN **5**

nicely illustrates the different behavior of the two functions. Isomer **4e** shows conversion to **5**, whereas **5** and **4f** are inert under the conditions and so is isonitrile **4g**. The lack of reactivity in **4g** suggests that reaction of **4e** proceeds not by substitution but by elimination–addition under activation by the cyano group. The *nitrile* group in **4e** and **5** is too low ranked to be expelled and **4f** is inert because the *isonitrile* group is neither a particularly good activating group nor a particularly good nucleofuge. Formation of an alkene from **4e** under basic conditions has been referred to previously but no details are available.[8]

Acknowledgment. We thank the SERC (CASE Studentship to B.A.J.), ICI Plant Protection, Dr. I. T. Kay, and UCNW for their support of this work and Gwerydd Griffiths for some preliminary studies.

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