The Biosynthesis of Thiamine. Syntheses of [1,1,1,5-$^2H_4$]-1-Deoxy-D-*threo*-2-pentulose and Incorporation of this Sugar in Biosynthesis of Thiazole by *Escherichia coli* Cells

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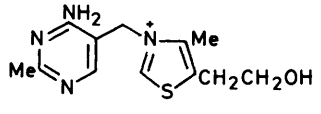
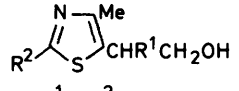
Non-growing, washed cells of *Escherichia coli*, depressed for the synthesis of thiamine, were incubated in the presence of [1,1,1,5-$^2H_4$]-1-deoxy-D-*threo*-2-pentulose (9) in a medium containing the pyrimidine moiety of thiamine, L-tyrosine, and glucose. The thiamine thus biosynthesized was extracted and cleaved to give 5-(2-hydroxyethyl)-4-methylthiazole (HET) which was examined as the trifluoroacetate derivative by electron-impact mass spectrometry. The distribution of the label in the fragments indicated that the pentulose (9) was a precursor of the C_5-chain of HET without C–C bond cleavage. Several routes to 1-deoxypentuloses are described. Condensation of 2,4-*O*-benzylidene-D-[4-$^2H_1$]threose (23) with trideuteriomethylmagnesium iodide gave the protected 1-deoxypentitols (24) and (25). Brominolysis of the mixed dibutylstannylidenes then afforded [1,1,1,5-$^2H_4$]-3,5-*O*-benzylidene-1-deoxy-D-*threo*-2-pentulose (26), which was converted into the free sugar (9) by acidic hydrolysis. 1-Deoxy-D-*erythro*-2-pentulose was prepared in similar manner. Condensation of 2-([$^2H_3$]-methyl)-1,3-dithian with 2,3-*O*-isopropylidene-D-glyceraldehyde, followed by a C-3 epimerization step also led, after deprotection, to a mixture of [1,1,1-$^2H_3$]-1-deoxy-D-*erythro*- and [1,1,1-$^2H_3$]-1-deoxy-D-*threo*-2-pentulose, (5) and (6).

THE present evidence for the biosynthesis of the thiazole moiety (2) (HET) of thiamine (1) may be summarized as follows. In *Enterobacteria*, C-2 of HET comes from C-2 of tyrosine,[1] which also supplies the nitrogen.[2] The fate of the carboxy-group of tyrosine is unknown, although it is present in a stray, obscure, non-precursor metabolite, 5-(2-hydroxyethyl)-4-methylthiazole-2-carboxylic acid (3).[3] The rest of the tyrosine molecule is excreted as 4-hydroxybenzyl alcohol.[4] More recently, independent investigations gave strong indications that a C_5-sugar might be the precursor of the C_5-chain in HET. In 1978, we discovered 5-(1,2-dihydroxyethyl)-4-methylthiazole (4) in *E. coli* culture media.[5] The glycol (4) is not a precursor of HET but it could be demonstrated that, starting from tyrosine, there was a common part to the biosynthetic pathway of both compounds.



(1)

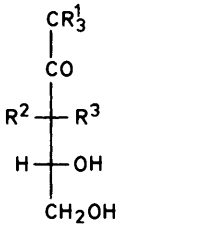


(2) $R^1 = R^2 = H$
(3) $R^1 = H, R^2 = CO_2H$
(4) $R^1 = OH, R^2 = H$

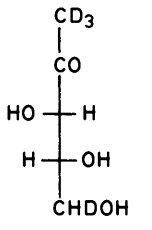
We therefore began to look for highly functionalized molecules as precursors of the C_5-chain of HET, and to consider the possible intermediacy of pentose sugars.[5] The pattern of incorporation of labelled glucose, pyruvate, and glyceraldehyde led R. H. White [6] to postulate the intermediacy of a pentulose derivative, arising by the reaction (1). Finally, R. L. White and I. D. Spenser [7]

$$CH_3COCO_2^- + CHO{\cdot}CHOH{\cdot}CH_2OPO_3H_2 \longrightarrow CH_3COCHOH{\cdot}CHOH{\cdot}CH_2OPO_3H_2 + CO_2 \quad (1)$$

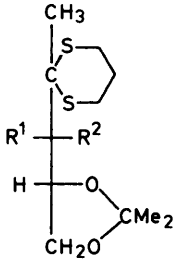
interpreted their results with yeast as an indication that a pentulose phosphate was the precursor. However, there may be only gross similarities in the biosyntheses of the C_5-chain of HET by *Enteriobacteria* and yeasts, for there is evidence of major differences in the way they build some other parts of the molecule. We then reported that the incorporation of three deuterium atoms from [1,1,1-$^2H_3$]-1-deoxy-D-*threo*-2-pentulose (6) into the methyl group of HET, even in the presence of excess of glucose, was a strong indication that this sugar was utilized without rupture in the building of the C_5-chain.[8] The D-*erythro*-isomer (5) was inactive in our system. We later gave a



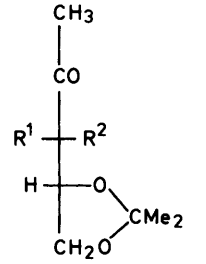
(5) $R^1 = D, R^2 = H, R^3 = OH$
(6) $R^1 = D, R^2 = OH, R^3 = H$
(7) $R^1 = R^2 = H, R^3 = OH$
(8) $R^1 = R^3 = H, R^2 = OH$



(9)



(10) $R^1 = H, R^2 = OH$
(11) $R^1 = OH, R^2 = H$



(12) $R^1 = H, R^2 = OH$
(13) $R^1 = OH, R^2 = H$

† Equipe de recherches associée au C.N.R.S. Nr. 479.

preliminary report [9] on the incorporation of 1-deoxy-D-*threo*-2-pentulose labelled at both ends. We now report, in final form, the syntheses of the precursors involved, compounds (5), (6), and (9), and biochemical investigations with the pentulose (9). One point of chemical interest is the easy access to 1-deoxypentuloses by the brominolysis of suitable stannylidene derivatives of 1-deoxypentitols. We also report the synthesis of the thiazoles (3) and (4).

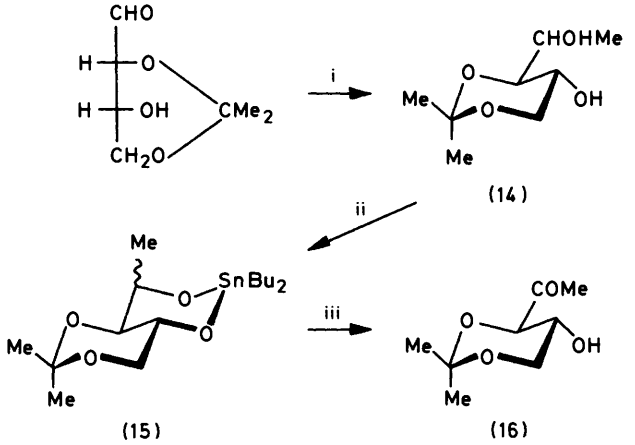
DISCUSSION

(a) *Chemical Syntheses.*—The labelled molecules which we needed were [1,1,1-$^2H_3$]-1-deoxy-D-*erythro*-2-pentulose (5), [1,1,1-$^2H_3$]-1-deoxy-D-*threo*-2-pentulose (6), and [1,1,1,5-$^2H_4$]-1-deoxy-D-*threo*-2-pentulose (9). Although 1-deoxy-D-*erythro*-2-pentulose (7) is a known compound,[10] it seemed that the reported synthesis would not easily lend itself to the preparation of labelled analogues, and we devised two other routes to this family of sugars.

The first begins with a 1,3-dithian condensation.[11] We recognised that the addition of the anion of acetaldehyde trimethylene dithioacetal to 2,3-*O*-isopropylidene-D-glyceraldehyde gave the practically pure D-*erythro*-isomer (10). This is the only configuration compatible with the fact that the protected sugar (12) was reduced and hydrolysed to a mixture containing the known [10] 1-deoxy-D-arabinitol. This preferential formation of the D-*erythro*-isomers is generally observed in the attack of 2,3-*O*-isopropylidene-D-glyceraldehyde by carbon nucleophiles. However, a *ca.* 1 : 1 mixture of the D-*erythro*- and D-*threo*-isomers, (10) and (11), could be obtained by the oxidation of derivative (10) to a ketone (72%) followed by borohydride reduction. This mixture could be resolved by column chromatography, but separation was more efficient at the next stage after generation of the carbonyl function by the boron trifluoride–diethyl ether–mercury(II) oxide procedure.[12] The ketones (12) and (13) thus obtained were hydrolysed to the free pentuloses (7) and (8) respectively. The same sequence of reactions was performed with [2,2,2-$^2H_3$]-acetaldehyde trimethylene dithioacetal, finally giving the labelled pentuloses (5) and (6). The correctness of the overall labelling procedure for the deuteriated analogues of the protected pentuloses (12) and (13) was ascertained by the absence of CH_3 signals in their ^1H n.m.r. spectra.

Our second route to 1-deoxy-2-pentuloses allows labelling with deuterium at both ends of the molecule. The key step is the oxidation of a diol to a hydroxy-ketone by brominolysis of its dibutylstannylidene derivative, a reaction discovered in this laboratory and demonstrated in many instances.[13] Thus, the protected 1-deoxy-D-*erythro*-2-pentulose (16) was prepared from 2,4-*O*-isopropylidene-D-erythrose *via* the intermediate (15), according to Scheme 1. Brominolysis of stannylidenes has so far been found to be regiospecific, giving only one hydroxy-ketone. It is fortunate that, in the present case, the desired methyl ketone was formed, in nearly quantitative yield, from the mixture of epimeric diols (14).

For the synthesis of 1-deoxy-D-*threo*-2-pentulose (8), the starting material was D-arabinose (Scheme 2). The protected D-threose (18) was prepared by the oxidation of the stannylidene derivative of the glycol (17) with (diacetoxy)iodobenzene. This unusual procedure [14] allowed the use of organic solvents in the absence of water or acids, and apparently gave fewer by-products.[15] The less polar of the two protected pentitols (19) and (20) obtained at the next step was formulated as the D-*xylo*-derivative (19). Hydrolysis gave a crystalline tetraol, m.p. 77—78 °C, $[\alpha]_D^{20}$ 0°, which was considered to be authentic 1-deoxy-D-xylitol (although this has been described as an oil [16a]) because the reported constants for 1-deoxy-D-lyxitol * are m.p. 103 °C, $[\alpha]_D^{20}$ +42°.[16b]



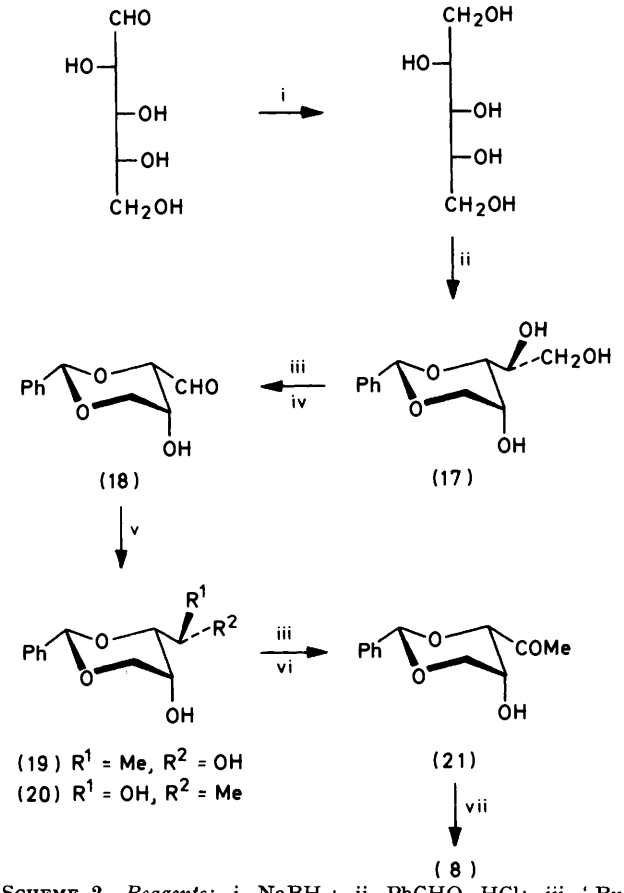
SCHEME 1 *Reagents*: i, MeMgI; ii, 'Bu_2SnO,' benzene; iii, Br_2, 4Å molecular sieves

The regiospecificity of the brominolysis of the mixed stannylidenes of the epimeric diols (19) and (20) was the same as above, leading to a 1-deoxy-D-*threo*-2-pentulose derivative. However, in this case the yield in 3,5-*O*-benzylidene-1-deoxy-D-*threo*-2-pentulose (21) was only 33%, from the mixture or either of its components. This was an unusually low yield for this kind of reaction, and much decomposition was observed. This may be a consequence of the *trans*-diaxial relationship of the 3-H and 4-OH moieties in the diols (19) and (20), which favours elimination. There is no such disposition in the diols (14).

The conformations drawn for compounds (16), (17), (20), and (21) are compatible with their ^1H n.m.r. spectra, which are reported in the Experimental section [for compounds (16), (20), and (21)].

Scheme 2 was easily adapted to the preparation of 1-deoxy-D-*threo*-2-pentulose labelled at both ends, compound (9), by using sodium [$^2H_4$]borohydride in step i and [$^2H_3$]iodomethane in step v. The labelled intermediates were then compounds (22)—(26). The tetraol [1-$^2H_1$]-D-arabinitol obtained in step i was a *ca.* 1 : 1 mixture of [1-$^2H_1$]-epimers. This was clearly shown in the ^1H n.m.r. spectra of the derived six-membered ring compounds, (22) and (26): two signals for 5-H were

* Systematic name: 5-Deoxy-D-arabinitol.

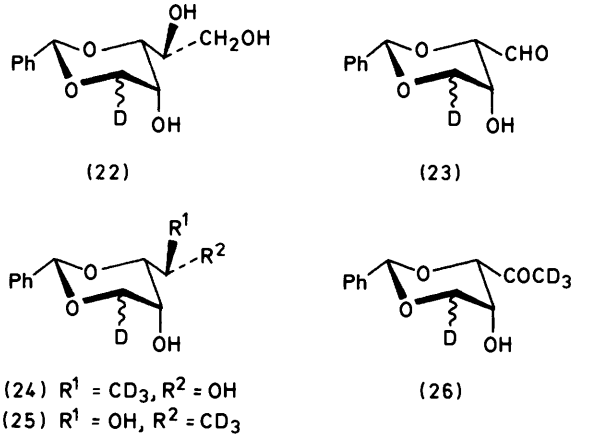


SCHEME 2 *Reagents*: i, $NaBH_4$; ii, PhCHO, HCl; iii, 'Bu_2^nSnO,' benzene; iv, $PhI(OAc)_2$; v, MeMgI; vi, Br_2, 4 Å molecular sieves; vii, AcOH, H_2O

observed, with a total intensity of one proton, corresponding to axial and equatorial hydrogen atoms at C-5. The large geminal coupling constant, $J_{5,5'}$ 11.5—13 Hz, present in the spectra of the undeuteriated analogues was absent from the spectra of these compounds.

The labile 2-pentulose (9) was prepared just before use by mild, acidic hydrolysis of the cyclic acetal (26).

(b) *Biochemical Experiments.*—Non-growing, washed cells of *Escherichia coli*, derepressed * for the biosynthesis of thiamine,[17] were incubated in the presence of the labelled pentulose (9) in a medium containing the pyrimidine moiety of thiamine, L-tyrosine, and glucose. The thiamine was then extracted and cleaved by hydrogen sulphite to give HET in the usual way. This was converted into the trifluoroacetate for m.s. examination.

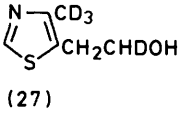


Incorporation of (5*RS*)-[1,1,1,5-$^2H_4$]-1-deoxy-D-*threo*-2-pentulose (9) into 5-(2-hydroxyethyl)-4-methylthiazole by *E. coli* [a]

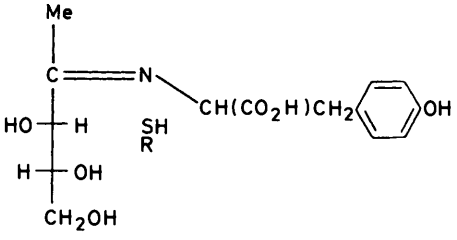
No. of ^2H atoms	Incorporation into		
		Fragment	
	M^+	A	B
0	100	100	100
1	2.7	0	3.3
2	3.6	3.3	0
3	4.9	9.8	26
4	22.3	28.7	0
5	1.7	0	0

[a] Data are presented as intensities of the peaks of labelled fragments relative to those of the unlabelled ones. For other details, see Experimental section, Biochemical Experiments.

The relevant fragmentations of HET trifluoroacetate are given on Scheme 3,[6] while the observed incorporations of deuterium are given in the Table. These figures indicate the presence of *ca.* 20% of a species with four deuterium atoms in the molecular ion and fragment (A),



(27)

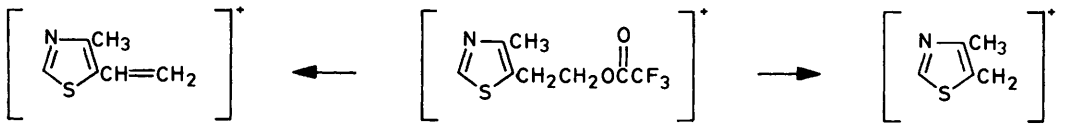


(C)

and three deuterium atoms in fragment (B). Now, fragment (A) retains the hydrogen atoms of the CH_3 and CH_2OH groups of HET, while in fragment (B) the CH_2OH group is lost. Thus, this fragmentation shows the presence in biosynthetic HET of the species (27).

From an estimation of the total amount of HET biosynthesized during the incubation, one can calculate that *ca.* 25% originated from the deoxypentulose (9). The distributions of the label in the C_5-chains of both the precursor (9) and HET indicated incorporation without rupture since otherwise fragments of compound

* 'Depressed cells' are grown in conditions such that their intracellular concentration of thiamine has been kept below the level where thiamine can repress the production of enzymes involved in its biosynthesis.



SCHEME 3

(9) would have mixed with the unlabelled precursors of the thrice-as-much abundant, unlabelled HET to give a completely different pattern.

A Schiff base (C) of tyrosine and 1-deoxy-D-*threo*-2-pentulose seems a probable intermediate, where the sulphur donor is probably cysteine.[18] Reaction of 1-deoxy-D-*threo*-2-pentulose, tyrosine or glycine, and hydrogen sulphide in dimethylformamide solution gave traces of the thiazoles (2) and (4), which could be detected by bioautography. The presence, in cells, of 1-deoxy-D-*threo*-2-pentulose or a phosphorylated derivative has not been recorded so far.

EXPERIMENTAL *

All chromatographic separations were performed on silica gel columns, with monitoring of fractions by thin-layer chromatography (t.l.c.) on silica gel plates. ^{1}H N.m.r. spectra were recorded at 250 MHz for samples dissolved in $CDCl_3$, in the presence of Me_4Si as internal reference, unless otherwise stated. The method for hydrogen analysis in deuteriated samples does not differentiate between isotopes, so the results are reported as g-atoms per 100 g of sample. Light petroleum refers to that fraction boiling in the range 40—65 °C.

1-*Deoxy*-4,5-O-*isopropylidene*-D-erythro-2-*pentulose Trimethylene Dithioacetal* (10).—n-Butyl-lithium (1.6M in hexane; 22 mmol) was added to a solution of 1,3-dithian (2.4 g) in tetrahydrofuran (THF) at −30 °C under nitrogen. After 45 min, iodomethane (2.84 g) was added dropwise. After 30 min, more n-butyl-lithium (22 mmol) was introduced and, after a further 45 min, 2,3-*O*-isopropylidene-D-glyceraldehyde [prepared from 1,2:5,6-di-*O*-isopropylidene mannitol (15 mmol)] was added. After a further 5 min, the mixture was poured into water (150 ml) and extracted with diethyl ether. Evaporation of the extract gave a crystalline residue which was washed with heptane and was then crystallized from hexane–ethyl acetate to give the *product*, (yield 1.8 g; 34% from iodomethane), m.p. 109 °C (Found: C, 50.0; H, 7.9; S, 24.1. $C_{11}H_{20}O_3S_2$ requires C, 50.0; H, 7.6; S, 24.3%).

1-*Deoxy*-4,5-O-*isopropylidene*-D-erythro- *and* 1-*Deoxy*-4,5-O-*isopropylidene*-D-threo-2-*pentulose Trimethylene Dithioacetal* (10) *and* (11).—To a mixture of dry dimethyl sulphoxide (1 g) and dichloromethane (6 ml) at −70 °C was added dropwise a solution of trifluoroacetic anhydride (1 ml) in dichloromethane (6 ml), followed by the protected pentulose (10) (1.28 g). After 5 min, triethylamine (2.6 ml) was added and the mixture was allowed to warm up to room temperature; it was then washed three times with water, and was then dried ($MgSO_4$) and evaporated to small volume. Flash chromatography (toluene–ethyl acetate, 9 : 1) gave a crude, oily mixture of ketones (0.926 g, 72%). This was dissolved in methanol and reduced with sodium borohydride (0.5 g) to give a 1 : 1 epimeric mixture of (10) and (11) which could be resolved by column chromatography (toluene–ethyl acetate, 4 : 1) into the less polar D-*erythro*-component (10) (Found: C, 50.0; H, 7.7; S, 24.3. $C_{11}H_{20}O_3S_2$ requires C, 50.0; H, 7.6; S, 24.3%) and the more polar D-threo-*component* (11) (Found: C, 49.7; H, 7.6; S, 24.1%).

1-*Deoxy*-4,5-O-*isopropylidene*-D-erythro- *and* 1-*Deoxy*-4,5-O-*isopropylidene*-D-threo-2-*pentulose* (12) *and* (13).—The mixture of protected pentuloses (10) and (11) (675 mg) was dissolved in THF (3 ml) and the solution was added dropwise to a stirred suspension of mercury(II) oxide (2.5 g) in a mixture of THF (6 ml), water (1 ml), and boron trifluoride–diethyl ether (0.6 ml). After completion of the addition, the mixture was poured into water (50 ml), and then filtered and washed with aqueous potassium carbonate (27 g in 30 ml). The THF solution was dried ($MgSO_4$) and volatile components were removed under reduced pressure. Chromatography of the residue (toluene–ethyl acetate, 13 : 7) gave first the D-erythro-isomer (12) as a liquid (250 mg); δ_H 1.35 and 1.50 (total 6 H, 2 × s, CMe_2), 2.35 (3 H, s, COMe), 3.50 (1 H, d, J 6 Hz, OH), and 4.00 (total 4 H, m, 3-, 4-, 5-, and 5-H).

Continued elution gave the D-threo-*isomer* (13) as a liquid (235 mg); δ_H 1.34 and 1.38 (total 6 H, 2 × s, CMe_2), 2.32 (3 H, s, COMe), 3.50 (1 H, d, J 4 Hz, OH), 4.10 (total 3 H, m, 3-, 5-, and 5-H), and 4.50 (1 H, dt, J 2.5, 6.5, and 6.5 Hz, 4-H) (Found: C, 54.9; H, 8.1. $C_8H_{14}O_4$ requires C, 55.2; H, 8.1%.

Sodium borohydride-reduction of the cyclic acetal (12) in methanol, followed by chromatography of the isolated reduction product (toluene–ethyl acetate, 3 : 1) on one hundred times its weight of silica gel gave, as the less polar component, a protected pentitol. This was hydrolysed in 80% acetic acid to 1-deoxy-D-arabinitol, m.p. 128—130 °C; $[\alpha]_D^{21}$ 0° (*c*, 1 in water) [lit.,[10] m.p. 130 °C; $[\alpha]_D^{21}$ 1.4° (*c*, 1.2 in water)].

1-*Deoxy*-D-erythro- *and* 1-*Deoxy*-D-threo-2-*pentulose* (7) *and* (8).—These were prepared just before use by heating a solution of the corresponding isopropylidene acetal in 1 : 4 water–acetic acid for 40 min at 80 °C and evaporating to dryness under reduced pressure. The optical rotations found were for isomer (7), $[\alpha]_D^{21}$ −30° (*c*, 1 in water) [lit.,[10] $[\alpha]_D^{21}$ −37° (*c*, 1.3 in water)], and for isomer (8), $[\alpha]_D^{21}$ +34° (*c*, 1 in water).

Sodium borohydride-reduction of the pentulose (7) gave (presumably) a mixture of 1-deoxy-D-arabinitol and 1-deoxy-D-ribitol which, however, moved as a single spot on t.l.c. [chloroform–methanol (3 : 2), R_F 0.40; butan-1-ol–acetic acid–diethyl ether–water (9 : 6 : 3 : 2), R_F 0.26].

[1,1,1-$^2H_3$]-1-*Deoxy*-D-erythro- *and* [1,1,1-$^2H_3$]-1-*Deoxy*-

* All benzylidene derivatives described are *S* at the Ph*C* carbon atom.

D-threo-2-*pentulose* (5) *and* (6).—These were prepared in identical manner to their unlabelled analogues (7) and (8), except that [$^2H_3$]iodomethane (2.84 g) was used in the first step. The COMe signals had vanished from the ^1H n.m.r. spectra of the [1,1,1-$^2H_3$] analogues of the acetals (12) and (13), but no other difference was noticeable.

2,4-O-*Isopropylidene*-D-*erythrose*.—4,6-*O*-Isopropylidene-D-glucopyranose (12.3 g),[19] which had been purified by column chromatography (diethyl ether–light petroleum, 1 : 1), was dissolved in water (250 ml). Sodium metaperiodate (24.02 g) was added in small portions, while keeping the pH of the stirred solution to a slightly basic value by the addition of saturated aqueous potassium carbonate. The mixture was kept for 2 h at room temperature and was then freeze-dried. Extraction of the residue with ethyl acetate gave a white solid (10 g), from which the protected tetrose was separated by chromatography (column 3.5 × 60 cm; chloroform–ethyl acetate, 1 : 1), (8.0 g; 90%); t.l.c. indicated the presence of two components (chloroform–ethyl acetate, 1 : 1); $\nu_{max.}$ 1 745 (CO, weak) and 3 220 and 3 490 cm^{-1} (OH).

1-*Deoxy*-3,5-O-*isopropylidene*-D-*ribitol* *and* 1-*Deoxy*-3,5-O-*isopropylidene*-D-*arabinitol* (14).—A solution of 2,4-*O*-isopropylidene-D-erythrose (9.0 g) in dry THF (100 ml) was added dropwise to a solution of the Grignard reagent prepared from iodomethane (12.2 ml) and magnesium (5.46 g) in diethyl ether (100 ml). The next day, methanol (10 ml) and ethyl acetate (150 ml) were added, the mixture was filtered over Celite and was then evaporated to dryness. The chloroform extract of the residue was purified by chromatography (diethyl ether as eluant) to give a mixture of the *pentitols* (14) (6.0 g; 61%) [t.l.c. (diethyl ether) R_F 0.30 and 0.47]; $\nu_{max.}$ 3 400br cm^{-1} (OH) (Found: C, 55.1; H, 9.1. $C_9H_{16}O_4$ requires C, 54.5; H, 9.1%).

1-*Deoxy*-3,5-O-*isopropylidene*-D-erythro-2-*pentulose* (16).—The dibutyltin derivatives (15) of the mixture of protected pentitols (14) (3.9 g) were prepared in the usual way with dibutyltin oxide (6.2 g) in benzene.[13] Part of the solvent was evaporated off, molecular sieves were added (4 Å; 4 g), and a solution of bromine (1.128 ml) in dry dichloromethane was added dropwise to the stirred mixture until a permanent pale-yellow colour was observed. The mixture was filtered and the filtrate was evaporated to dryness. Chromatography of the residue (column 4 × 60 cm; dichloromethane–ethyl acetate, 3 : 1) gave the *protected pentulose* (16) (3.5 g; 91%); R_F 0.43 (dichloromethane–ethyl acetate, 7 : 3); $[\alpha]_D^{20}$ +40.5° (*c*, 7 in dichloromethane); $\nu_{max.}$ 1 720 (CO) and 3 450 cm^{-1} (OH); δ_H 1.47 and 1.52 (total 6 H, 2 × s, CMe_2), 2.27 (3 H, s, COMe), 3.40 (1 H, s, OH), 3.63 (1 H, dd, $J_{4.5}$ 9 and $J_{5.5'}$ 10.5 Hz, 5-H), 3.77 (1 H, m, 4-H), 3.90 (1 H, dd, $J_{4.5'}$ 5 Hz, 5′-H), and 3.95 (1 H, d, $J_{3.4}$ 8.5 Hz, 3-H).

Benzoylation (benzoyl chloride, pyridine) and chromatographic purification (dichloromethane–ethyl acetate, 4 : 1) gave the 4-*benzoate* as a gum, $[\alpha]_D^{20}$ −92° (*c*, 7.2 in methanol) (Found: C, 64.5; H, 6.4. $C_{15}H_{18}O_5$ requires C, 64.7; H, 6.5%).

2,4-O-*Benzylidene*-D-*threose* (18).—The dibutyltin derivative of 1,3-*O*-benzylidene-D-arabinitol (17)[20] (25.5 g) was prepared in the usual way in benzene. The solution was concentrated to a volume of *ca.* 200 ml and was then cooled to 0 °C. 4 Å Molecular sieves (10 g) were added, followed by solid (diacetoxyiodo) benzene (34.2 g) in small portions, over 30 min, to the stirred mixture. The mixture was allowed to warm up to room temperature (16 h), then was filtered over Celite and the filtrate was evaporated to dryness. Chromatography of the residue (column 4.5 × 100 cm; chloroform–ethyl acetate, 7 : 3, with 2% methanol) gave the cyclic acetal (18) as a foam (21.5 g, 97%).

3,5-O-*Benzylidene*-1-*deoxy*-D-*xylitol* *and* 3,5-O-*Benzylidene*-1-*deoxy*-D-*lyxitol* * (19) *and* (20).—A solution of the aldehyde (18) (9.2 g) in dry THF (100 ml) was added dropwise during 1 h to a solution of the Grignard reagent prepared from iodomethane (18.7 g) and magnesium (4.3 g) in diethyl ether (100 ml), cooled in an ice-bath. The mixture was then kept for 16 h at room temperature and then poured into aqueous ammonium chloride. Extraction with dichloromethane (4 × 150 ml), followed by the usual work-up, gave a solid residue (9.2 g) which was crystallized from cyclohexane–ethyl acetate to give the D-xylo-*acetal* (19), m.p. 163 °C; $[\alpha]_D^{20}$ −3° (*c*, 2.5 in pyridine); R_F 0.25 (chloroform–ethyl acetate, 3 : 2, with 2% methanol); δ_H (250 MHz; $CDCl_3$–C_5D_5N, Me_4Si) 1.40 (3 H, d, $J_{1.2}$ 6.5 Hz, Me), 3.67 (1 H, dd, $J_{2.3}$ 6.8, $J_{3.4}$ 1 Hz, 3-H), 4.07 (total 2 H, m, $J_{5.5'}$ 11.5 and $J_{4.5'}$ 1 Hz, 4- and 5′-H), 4.32 (total 2 H, m, $J_{4.5}$ 2 Hz, 2- and 5-H), 5.5br (2 H, 2 × OH), 5.62 (1 H, s, PhC*H*), and 7.30 and 7.52 (total 5 H, Ph) (Found: C, 64.1; H, 7.1. $C_{12}H_{16}O_4$ requires C, 64.3; H, 7.2%).

The mother-liquors were evaporated to dryness. Chromatography of the residue (column 3.5 × 60 cm; chloroform–ethyl acetate, 7 : 3, with 2% methanol) gave the D-lyxo-*acetal* (20) as a glass (3.42 g; 35%); $[\alpha]_D^{20}$ +10.3° (*c*, 5.5 in methanol); δ_H † 1.2 (3 H, $J_{1.2}$ 6.5 Hz, Me), 2.97br and 3.32br (total 2 H, 2 × s, 2 × OH), 3.40 (1 H, d, $J_{2.3}$ 7 Hz, 3-H), 3.41 (1 H, s, 4-H), 3.87 (1 H, d, $J_{5.5'}$ 11.5, 5′-H), 3.97 (1 H, t, 2-H), 4.10 (1 H, d, 5-H), 5.49 (1 H, s, PhC*H*), and 7.32 and 7.45 (total 5 H, Ph) (Found: C, 64.2; H, 7.2. $C_{12}H_{16}O_4$ requires C, 64.3; H, 7.2%).

1-*Deoxy*-D-*xylitol*.—A solution of the cyclic acetal (19) in 70% aqueous acetic acid was heated at 80 °C for 1 h, then evaporated to dryness. The residue was co-evaporated several times with propan-2-ol–toluene, and then kept *in vacuo* at room temperature, when it slowly crystallized (3.0 g), m.p. 77—78 °C (from 95% ethanol); $[\alpha]_D^{20}$ 0° (*c*, 5 in water) (Found: C, 39.5; H, 8.4. $C_5H_{12}O_4 \cdot H_2O$ requires C, 39.9; H, 9.1%).

3,5-O-*Benzylidene*-1-*deoxy*-D-threo-2-*pentulose* (21).—The mixed dibutyltin derivatives of the cyclic acetals (19) and (20) (2.36 g) were prepared in benzene (the period of refluxing was restricted to 1 h to avoid discolouration), the solution was concentrated to *ca.* 80 ml, and then cooled to room temperature. 4 Å Molecular sieves were added (4 g). To the well stirred solution, a solution of bromine (0.5 ml) in dry dichloromethane (10 ml) was added dropwise until a persistent red colour was observed. The solution was then filtered over Celite and the filtrate was evaporated to dryness. Chromatography of the residue (column 3.6 × 60 cm; chloroform–ethyl acetate, 1 : 1) gave the *protected pentulose* (21) as a slowly crystallizing gum (0.77 g; 33%), m.p. 97 °C; $[\alpha]_D^{20}$ −89° (*c*, 2 in methanol); R_F 0.43 (chloroform–ethyl acetate, 3 : 2, with 2% methanol); $\nu_{max.}$ 1 720 (CO) and 3 450 cm^{-1} (OH); δ_H 2.28 (3 H, s, Me), 3.00 (1 H, s, OH), 4.00br (1 H, 4-H), 4.08 (1 H, dd, $J_{4.5'}$ 1.2 and $J_{5.5'}$ 12 Hz, 5′-H), 4.20 (1 H, dd, $J_{4.5}$ 1.5 Hz, 5-H), 4.31 (1 H, d, $J_{3.4}$ 1.5 Hz, 3-H), 5.58 (1 H, s, PhC*H*), and 7.38 and 7.65 (total 5 H, Ph); *m/z* (relative intensities) 221 [$(M - H)^+$, 1], 180 [$(M - C_2H_2O)^+$, 15] 179 [$(M - Ac)^+$, 100],

* Systematic name: 1,3-*O*-Benzylidene-5-deoxy-D-arabinitol.

† Non-systematic numbering scheme.

107 ($PhCH_2O$, 74), 106 (PhCHO, 10), 105 (PhCO, 26), 77 (Ph, 27), and 43 (Ac, 79) (Found: C, 64.6; H, 6.4. $C_{12}H_{14}O_4$ requires C, 64.8; H, 6.4%).

Acetylation (pyridine–acetic anhydride) gave 4-O-*acetyl*-3,5-O-*benzylidene*-1-*deoxy*-D-threo-2-*pentulose*, m.p. 100 °C; $[\alpha]_D^{20}$ −101° (*c*, 2 in methanol); $\nu_{max.}$ 1 720 (ketone) and 1 740 cm^{-1} (acetyl); δ_H 2.07 (3 H, s, C·COMe), 2.33 (3 H, s, OAc), 4.08 (1 H, dd, $J_{5,5'}$ 13 and $J_{4,5'}$ 1.5 Hz, 5′-H), 4.39 (total 2 H, m, $J_{4,5}$ 1.5 and $J_{3,4}$ 2.5 Hz, 3- and 5-H), 5.06 (1 H, m, 4-H), 5.60 (1 H, s, PhC*H*), and 7.38 and 7.52 (total 5 H, Ph) (Found: C, 63.8; H, 6.0. $C_{14}H_{16}O_5$ requires C, 63,6; H, 6.1%).

Mild, acidic hydrolysis (80% acetic acid) of the cyclic acetal (21), followed by borohydride reduction, gave (presumably) a mixture of 1-deoxy-D-lyxitol * and 1-deoxy-D-xylitol which moved as one spot on silica gel t.l.c. with two different developers: chloroform–methanol, 3 : 2 (R_F 0.4) and butan-1-ol–acetic acid–diethyl ethyl–water, 9 : 6 : 3 : 2 (R_F 0.26). The product obtained on analogous treatment of the cyclic acetal (13) behaved identically. On the other hand, the mixture of 1-deoxy-D-ribitol and 1-deoxy-D-arabinitol prepared from the cyclic acetal (12) showed R_F 0.47 and 0.32, respectively, with the two aforementioned developers.

(1RS)-[1-$^2H_1$]-D-*Arabinitol*.—Sodium borodeuteride (3.0 g) was added in small portions during 1 h to a solution of D-arabinose (21 g) in water (150 ml) containing potassium hydrogen carbonate (10 g). The next day the solution was de-ionized on a Dowex-50 (H^+) column (4 × 40 cm), and then evaporated to dryness. Repeated co-evaporation of the residue with methanol, followed by crystallization from the same solvent, gave [1-$^2H_1$]-D-*arabinitol* (19.8 g; 91%), m.p. 100 °C; $[\alpha]_D^{20}$ 0° (*c*, 6 in water); $\nu_{max.}$ 2 150 and 2 190 cm^{-1} (CD) (Found: C, 39.2. $C_5H_{11}DO_5$ requires C, 39.2%) [(H-isotopes) Found: 7.8. $C_5H_{11}DO_5$ requires 7.9 g-atom%].

(1RS)-1,3-O-*Benzylidene*-D-[1-$^2H_1$]*arabinitol* (22).—Hydrogen chloride was passed for 20 min into a suspension of finely ground D-[1-$^2H_1$]arabinitol (35 g) in benzaldehyde (30 ml), after which dissolution was complete. After 18 h, the mixture was pulverised, kept for 1 d *in vacuo* over potassium hydroxide and sulphuric acid, and then washed in turn with diethyl ether, dilute aqueous sodium hydrogen carbonate, and water. The residue was crystallized from ethanol, to which had been added a few drops of concentrated aqueous ammonia, to give the *labelled cyclic acetal* (22) (38.16 g; 72%) (*cf.* ref. 19), m.p. 150 °C; $[\alpha]_D^{20}$ −8.2° (*c*, 3.5 in pyridine); δ_H (400 MHz, C_5D_5N, exchange of labile ^1H with ^2H) 4.18 (0.5 H, d, $J_{1,2}$ 1.5 Hz, 1-H), 4.50 (0.5 H, d, $J_{1,2}$ 1.5 Hz, 1′-H), 4.28 (1 H, dd, $J_{4,5'}$ 5.5 and $J_{5,5'}$ 11.5 Hz, 5′-H), 4.39 (total 2 H, m, 3- and 5-H), 4.42 (1 H, dd, $J_{1',2}$ 1.5 and $J_{2,3}$ 9 Hz, 2-H), 4.74 (1 H, m, 4-H), 5.82 (1 H, s, PhC*H*), and 7.35 and 7.70 (total 5 H, Ph) (Found: C, 59.6. $C_{12}H_{15}DO_5$ requires C, 59.7%) [(H-isotopes) Found: 6.7. $C_{12}H_{15}DO_5$ requires 6.6 g-atom%].

(4RS)-2,4-O-*Benzylidene*-D-[4-$^2H_1$]*threose* (23).—This was prepared in identical manner to the analogue (18); $\nu_{max.}$ 1 730 (CO, weak) and 3 410 cm^{-1} (OH) (Found: C, 62.9. $C_{11}H_{11}DO_4$ requires C, 63.1%) [(H-isotopes) Found: 5.7. $C_{11}H_{11}DO_4$ requires 5.7 g-atom%].

(5RS)-[1,1,1,5-$^2H_4$]-3,5-O-*Benzylidene*-1-*deoxy*-D-*xylitol* *and* (5RS)-[1,1,1,5-$^2H_4$]-3,5-O-*Benzylidene*-1-*deoxy*-D-*lyxitol* † (24) *and* (25).—The mixture of the pentitols (24) and (25) was prepared in analogous manner to the unlabelled derivatives (19) and (20), starting from the aldehyde (23) and [$^2H_3$]iodomethane. Only the D-*xylo*-isomer (24) was isolated in the pure state by crystallization from the crude mixture, m.p. 150—158 °C; $[\alpha]_D^{20}$ 0° (*c*, 2 in methanol); $\nu_{max.}$ 2 210 (CD, weak) and 2 350 cm^{-1} (OH); δ_H 3.60 (1 H, dd, $J_{2,3}$ 7 and $J_{3,4}$ 1.5 Hz, 3-H), 3.96 (1 H, dd, $J_{4,5}$ 3 Hz, 4-H), 4.16 (1 H, d, 2-H), 4.25 (1 H, s, 5-H), 5.42br (total 2 H, 2 × s, 2- and 4-OH), 5.57 (0.5 H, s, PhC*H*), and 7.28 and 7.45 (total 5 H, Ph).

(5RS)-[1,1,1,5-$^2H_4$]-3,5-O-*Benzylidene*-1-*deoxy*-D-threo-2-*pentulose* (26).—This was prepared in the same way as the unlabelled analogue (21), starting from the mixture of protected pentitols (24) and (25), in 33% yield, m.p. 97 °C (from ethyl acetate–cyclohexane); $[\alpha]_D^{20}$ −90° (*c*, 2.2 in methanol); $\nu_{max.}$ 1 715 (CO), 2 140 and 2 210 (CD), and 3 400 cm^{-1} (OH); δ_H 2.95 (1 H, d, *J* 10.5 Hz, OH), 3.99 (1 H, m, $J_{3,4}$ 10.5 Hz, 4-H), 4.05 (0.5 H, d, $J_{4,5}$ 1 Hz, 5-H), 4.28 (0.5 H, d, $J_{4,5'}$ 1 Hz, 5′-H), 4.31 (1 H, d, $J_{3,4}$ 1.5 Hz, 3-H), 5.58 (1 H, s, PhC*H*), and 7.38 and 7.52 (total 5 H, Ph); *m/z* (rel. int.) 225 [(*M* − H)$^+$, 0.4], 181 (20), 180 [(*M* − CD_3CO)$^+$, 100], 107 [(PhCHOH)$^+$, 72], 106 (8), 105 ($PhCO^+$, 27), 77 (Ph, 26), and 46 (CD_3CO^+, 64) (Found: C, 63.5. $C_{12}H_{10}D_4O_4$ requires C, 63.7%) [(H-isotopes) Found: 6.2. $C_{12}H_{10}D_4O_4$ requires 6.2 g-atom%].

(5RS)-[1,1,1,5-$^2H_4$]-1-*Deoxy*-D-threo-2-*pentulose* (9).—A solution of the cyclic acetal (26) in 80% aqueous acetic acid was heated at 80 °C for 40 min and then evaporated to dryness to give the *free, labelled pentulose* (9) as a gum which was utilized immediately in the biochemical experiments (see below).

rac-5-(1,2-*Dihydroxyethyl*)-4-*methylthiazole* (4).—A solution of potassium permanganate (5.3 g) and magnesium sulphate heptahydrate (4.1 g) in water (125 ml) was slowly added to a solution of 4-methyl-5-vinylthiazole [21] (6.25 g) in acetone (60 ml) at 0 °C. After 1 h manganese dioxide was filtered off, the acetone was evaporated off, and the residue was dissolved in water. Continuous extractions with diethyl ether gave the *glycol* (80%) m.p. 102 °C (from ethyl acetate); $\lambda_{max.}$ (ethanol) 249 nm (ε 4 500); $\lambda_{max.}$ (1M HCl) 256 nm (ε 4 700); δ_H ([$^2H_5$]pyridine) 2.10 (3 H, s, Me), 3.80 (2 H, d, *J* 6 Hz, CH_2OH), 5.00 [1 H, t, C*H*(OH)], 6.40 (2 H, s, 2 × OH), and 8.50 (1 H, s, 2-H) (Found: C, 45.3; H, 5.6; N, 8.8. $C_6H_9NO_2S$ requires C, 45.3; H, 5.7; N, 8.8%).

5-(2-*Hydroxyethyl*)-4-*methylthiazole*-2-*carboxylic Acid* (3).—A solution of phenyl-lithium in hexane (13 ml; 23.4 mmol) was added dropwise to a solution of 5-(2-hydroxyethyl)-4-methylthiazole (2) (HET) (Aldrich) (10 mmol) in dry THF (50 ml) under nitrogen at room temperature. After 1 h the viscous, pink solution was transferred, with a nitrogen overpressure, to a flask containing an excess of solid CO_2. When all the carbon dioxide had evaporated, water (50 ml) and diethyl ether (50 ml) were added to the residue. The aqueous phase was separated, extracted several times with dichloro- methane, and evaporated to dryness. Ethanol (4 ml) was added to the residue and, after filtration, acetone was added to the ethanolic solution to precipitate the *lithium salt* as a very hygroscopic powder (58%).

Ion exchange with Dowex 50 (cyclohexylammonium) resin gave the *cyclohexylammonium salt* as needles, m.p. 173 °C (from ethanol–acetone, 1 : 2); $\lambda_{max.}$ (water, pH 7) 288 nm (ε 7 900) (Found: C, 54.3; H, 7.6; N, 9.8; S, 11.0.

* Systematic name: 5-deoxy-D-arabinitol.

† Systematic name: (1*RS*)-[1,5,5,5-$^2H_4$]-1,3-*O*-Benzylidene-5-deoxy-D-arabinitol.

$C_{13}H_{22}N_2O_3S$ requires C, 54.5; H, 7.7; N, 9.8; S, 11.2%).

Elution with 2M acetic acid from a Dowex 2 (acetate) column gave the free acid as tiny crystals, m.p. 100 °C (from water–acetone, 1 : 5).

Esterification (CH_2N_2) gave the methyl ester, m.p. 92 °C (from toluene); δ_H 2.40 (3 H, s, 4-Me), 3.05 (2 H, t, J 6 Hz, CCH_2C), 3.90 (2 H, t, CH_2OH), and 3.95 (3 H, s, OMe).

Biological Experiments.—Derepression of thiamine biosynthesis and incubation. A shikimate auxotroph of *E. coli* (strain 83-1) was cultivated according to a previously described procedure [1] in the presence of adenosine. The cells were washed and resuspended in a minimal medium [1] without glucose. The suspension (105 ml, dry weight of cells 2 mg/ml) was added to the labelled pentulose (9) (0.46 mmol) in water (5 ml) and the mixture was stirred for 5 min at 37 °C. The pyrimidine moiety of thiamine (50 μg) L-tyrosine (sufficient to make a mixture of 0.2mM), and glucose (0.4%) were added and the suspension was stirred again for 1 h at 37 °C and then centrifuged (4 000 g, 15 min).

Extraction and purification of thiamine. The cells were resuspended in 0.02M acetate buffer (pH 5; 15 ml) and heated for 20 min at 100 °C. To hydrolyse the phosphate esters of thiamine, the suspension was incubated in the presence of an enzymatic preparation containing phosphatases (Mylase SR) (250 mg) for 4 h at 45 °C. After centrifugation (30 000 g, 30 min), the crude extract was fractionated on an Amberlite CG 50 (H^+) Column (0.7 × 10 cm); thiamine was recovered with 0.1M HCl (50 ml) as eluant and was purified further by linear gradient elution on a Dowex 50 (H^+) column (1 cm × 16 cm) with 1.1—5.4M HCl as eluant. Elution was followed fluorimetrically after oxidation of aliquots of the fractions to thiochrome.

Isolation of HET trifluoroacetate. Purified thiamine was cleaved [21] by hydrogen sulphite to HET and pyrimidinesulphonic acid. From the hydrogen sulphite solution (2 ml) adjusted to pH 7—8, HET was extracted with dichloromethane (5 × 2 ml). The solvent was evaporated off at 30 °C under a stream of nitrogen. The crude thiazole was taken up in a mixture of carbon tetrachloride (50 μl) and trifluoroacetic anhydride (5 μl); after 5 min, phosphate buffer (0.5M; pH 6; 0.5 ml) was added and the mixture was thoroughly stirred. The organic (lower) layer was recovered (*via* a syringe) immediately before injection into the gas-chromatography column.

Gas chromatography–Mass spectrometry of HET trifluoroacetate. A Ribermag R 10–10 gas chromatograph–mass spectrometer was used, equipped with a 25 m × 0.34 mm Girdel capillary column packed with CPSIL 5. The oven was at 150 °C. Under these conditions the retention time of HET trifluoroacetate was 1.5 min. The mass spectra were recorded after ionization by electron impact at 70 eV. The number of deuterium atoms in each fragment of interest was calculated from the average intensity of the mass peaks from all GC eluant fractions of HET trifluoroacetate. Results are given in the Table.

Assay of thiamine. Thiamine was assayed fluorimetrically after oxidation to thiochrome by mercury(II) chloride according to Morita and his co-workers.[23] The assay was performed on both an extract of non-incubated cells and on an aliquot of the extract of incubated cells.

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