

yield 0.75 g. (80%), m.p. 116–118° (preliminary softening). Pure material was obtained after several recrystallizations from ethanol-ether-petroleum ether; m.p. 127–129° (preliminary softening), $[\alpha]_D^{25} +172^\circ$ (*c* 0.5, water); X-ray powder diffraction data¹⁶: 12.8m, 8.29s(3), 6.97w, 6.42w, 4.88s(1), 4.33m, 4.13s(2), 3.93m, 3.31vw, 3.19w, 2.85vw, 2.26vw, 2.21vw, 2.14vw.

Anal. Calcd. for $C_9H_{17}NO_4S$: C, 45.91; H, 7.28; N, 5.95; S, 13.62. Found: C, 45.94; H, 7.20; N, 5.82; S, 13.41.

2-Acetamido-2-deoxy- β -L-arabinose (IV).—An aqueous solution of 693 mg. of mercuric chloride in 60 ml. of water, at 50°, was added to a solution of 300 mg. of ethyl 2-acetamido-2-deoxy-1-thio- β -L-arabinofuranose (III) in 15 ml. of water and allowed to stand at room temperature for 5 hr. The white precipitate which formed was removed by filtration. The filtrate was passed through a column (200 × 18 mm., diam.) of Amberlite MB-3¹⁵ and the column was washed with 200 ml. of water. The effluent and washings were concentrated to a sirup which was further dried by evaporation repeatedly with ethanol under reduced pressure. The product crystallized from methanol-acetone-ether; yield 158 mg. (65%), m.p. 151–155° (preliminary softening). Pure material was obtained by recrystallization from the same solvents. The compound was strongly reducing and exhibited a positive Morgan-Elson¹⁰ test for *N*-acetyl amino sugars; m.p. 154–156° (preliminary softening), $[\alpha]_D^{25} +147.5^\circ$ (initial, extrapolated) $\rightarrow +94^\circ$ (*c* 1, water, final);

(16) Interplanar spacing, Å., $CuK\alpha$ radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. First three strongest lines are numbered, strongest (1), repeated numbers indicate approximate equality.

X-ray powder diffraction data¹⁶: 9.03m, 7.99s(2), 7.01w, 4.76s(1), 4.60m, 4.39m, 4.10s(3), 3.53s(2), 3.33vw, 3.15w, 3.03m, 2.82w, 2.66vw, 2.48vw, 2.40vw, 2.23vw, 2.18vw, 2.07vw.

Anal. Calcd. for $C_7H_{13}NO_5$: C, 43.98; H, 6.85; N, 7.33. Found: C, 44.04; H, 6.85; N, 7.39.

2-Amino-2-deoxy- β -L-arabinose Hydrochloride (V).—An amount of 88 mg. of 2-acetamido-2-deoxy- β -L-arabinose (IV) was heated with 2 ml. of 4 *N* hydrochloric acid in a boiling water-bath for 30 min. The hydrolyzate was concentrated to a sirup and further dried by evaporation with methanol-acetone under reduced pressure, after which treatment the sirup crystallized; yield 78 mg. (89%), m.p. 150–154° dec. Pure material was obtained upon recrystallization from methanol-acetone; m.p. 153–155° dec., $[\alpha]_D^{25} +174$ (initial, extrapolated) $\rightarrow 115^\circ$ (*c* 0.5, water, final); X-ray powder diffraction data¹⁶: 7.67w, 6.20m, 5.20m, 4.72s(3), 4.11s(1), 3.83s(2), 3.36vw, 3.15w, 2.88w, 2.67vw, 2.50vw.

Anal. Calcd. for $C_5H_{12}ClNO_4$: C, 32.36; H, 6.52; N, 7.55; Cl, 19.10. Found: C, 32.59; H, 6.52; N, 7.46; Cl, 18.84.

The substance was strongly reducing and gave a strong positive reaction to the Elson-Morgan¹¹ and ninhydrin¹² color tests. In the Dische-Borenfreund test a maximum was developed at λ 490 μ .^{8,13} 2-Amino-2-deoxy- β -L-arabinose hydrochloride was obtained in the same way by the hydrolysis of ethyl 2-acetamido-2-deoxy-1-thio- β -L-arabinofuranoside with 4 *N* hydrochloric acid. The constants were identical with those reported above.

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COMMUNICATIONS TO THE EDITOR

A STEREOSPECIFIC DIHYDROLIPOIC DEHYDROGENASE FROM *SPINACIA OLERACEA*

Sir:

The oxidation of α -keto acids takes place in a number of integrated steps of which the last two are mediated by dihydrolipoic transacetylase and dihydrolipoic dehydrogenase.¹ The purification and properties of the dihydrolipoic dehydrogenase from animals and microorganisms have been described.^{2,3,4,5,6} Diaphorase was also found to have powerful dihydrolipoic dehydrogenase activity.⁷ Recently the involvement of flavoprotein in the pyruvate oxidase complex of *E. coli*⁸ and of diaphorase in α -ketoglutarate oxidase system of pig heart⁹ have been demonstrated. In contrast to dihydrolipoic transacetylase, dihydrolipoic dehydrogenase does not exhibit optical specificity.^{1,5}

A stereospecific dihydrolipoic dehydrogenase

which has been purified 30–40-fold from the acetone powder of *Spinacia oleracea* is DPN-linked and displays maximum activity at pH 8.0. The rate of reaction with dihydrolipoamide is faster than that with dihydrolipoic acid (Table I), as observed by Sanadi and Searls.⁴ The enzyme is active with (–)-dihydrolipoic acid (Table I). The slight activity observed with (+)-dihydrolipoic acid may be due to the contamination with the other isomer.

TABLE I

Substrate	DPNH formed in 3 min. (μ M.)
(±) Dihydrolipoic acid	0.044
(–) Dihydrolipoic acid ^a	0.033
(+) Dihydrolipoic acid ^a	0.005
(±) Dihydrolipoamide ^a	0.084
Cysteine	0
Reduced glutathione	0

^a Prepared from the oxidized compounds by the sodium borohydride reduction method of Wagner, *et al.*¹⁰

The incubation was carried out at 25° in 1.5 ml. containing 70 μ M. TRIS (pH 8.0), 0.5 μ M. DPN, 2 μ M. of substrate and 30 μ g. of dihydrolipoic dehydrogenase.

Though the reaction is freely reversible with lipoamide it has not been possible to demonstrate

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the reversibility of the reaction with lipoic acid as substrate. These results are in conformity with those of Gunsalus³ and Goldman⁶ and in partial agreement with those of Sanadi and co-workers.^{4,5}

Results of the stoichiometric experiments are shown in Table II. Since even by coupling with lactic dehydrogenase or alcohol dehydrogenase not more than 50% of the (\pm)-dihydrolipoic acid was oxidized, the stereospecificity of the enzyme was suspected. ($-$)-Dihydrolipoic acid was not fully oxidized, however, which may be due to contamination with the other isomer.

TABLE II

Substrate	μ M. added	μ M. oxidized
(\pm)-Dihydrolipoic acid	2.0	0.9
($-$)-Dihydrolipoic acid	2.0	1.7

The incubation was carried out at 25° for 30 min. in 1.5 ml. containing 60 μ M TRIS (*p*H 8.0), 5 μ M. DPN, 2 μ M. of substrate and 39 μ g. of dihydrolipoic dehydrogenase. Aliquots were assayed for SH groups¹¹

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(11) H. Beinert, unpublished method.

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THE POLYBORANE CARBONYL B₄H₈CO

Sir:

When borine carbonyl (BH₃CO) was discovered, a less volatile carbonyl also was observed.¹ Its formula now has been indicated by the virtually quantitative synthesis B₅H₁₁ + 2CO → B₄H₈CO + BH₃CO, and confirmed by analysis. This complete formation of B₄H₈CO and BH₃CO strongly suggests that the most typical reactions of B₅H₁₁ begin with cleavage into B₄H₈ and BH₃ fragments. The removal of one BH₃ from an open corner of the B₅H₁₁ structure² would leave B₄H₈ having the shape of a B₄H₁₀ molecule² with two hydrogen atoms missing. This pattern might help to explain why B₅H₁₁ so readily absorbs hydrogen to form B₄H₁₀ and B₂H₆.³

Synthesis.—The reactions summarized in Table I occurred in sealed 50-ml. bomb tubes at 22–27°. In Expts. 3 and 4 all CO was accounted for before and after the reaction; and CuO combustion demonstrated the absence of hydrogen. The B₅H₁₁

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had been purified by thorough fractionation (microcolumn with -78° reflux); vapor tension 52.8 mm. at 0° and m.p. -123.1° , showing the absence of B₅H₉.^{3,4} Its gas-volume may have been slightly underestimated. The B₄H₈CO also was reflux-fractionated, delivering only slight traces of more and less volatile material.

TABLE I

Expt. No.	B ₅ H ₁₁ (mmoles)	SYNTHESIS OF B ₄ H ₈ CO				
		Pressure of CO (atm.)	Time at 22–27° (hr.)	B ₄ H ₈ CO (mmoles)	BH ₃ CO (mmoles)	CO Consumed (mmoles)
1	3.7	7.0	4.0	1.6	3.4	..
2	0.72	19.5	16.0	0.54	0.61	..
3	1.35	19.8	1.92	1.32	1.36	2.6
4	1.08	20.0	2.50	1.07	1.15	2.3

The long-run Expts. 1 and 2 gave much non-volatile solid. The trace of such material from Expt. 4 was dissolved in nitric acid and the boron determined as 0.067 mmole (1.4%).

Physical Properties.—The gas-phase mol. wt. of B₄H₈CO was 77.9 or 79.4 (calcd., 79.35). Its vapor tensions (Table II) gave the equation $\log p_{\text{mm}} = 4.555 - 0.00340T + 1.75 \log T - 1649/T$; b.p. 59.6°; Trouton constant, 21.0 cal./deg. mole. Crystallization difficulties sometimes prevented m.p. determinations; however, two apparently reliable results (Stock plunger method) were -114.4 and -114.6°

TABLE II

<i>t</i> , °C.	VAPOR TENSIONS OF B ₄ H ₈ CO					
	-76.3	-72.1	-58.8	-43.1	-37.0	0.00
<i>p</i> _{mm} (obsd.)	0.34	0.49	1.61	5.47	8.5	71.2
<i>p</i> _{mm} (calcd.)	0.33	0.50	1.64	5.49	8.4	71.3

Analyses.—We found no single quantitative reaction that would give a complete elementary analysis, but separate determinations, based upon different reactions, verified the formula B₄H₈CO. A 60% dissociation at room temperature gave 96% of the expected CO, but the remaining sample was uncertain. Complete decomposition (Vycor tube, 800–900°) gave 81% of the expected CO, raised to 85% by pure-O₂ combustion of the non-volatile residue. Boron was estimated as 4.04 B per molecule, but hydrogen ran low. Bromine oxidation at 100° gave 0.81CO, 8.03 (\pm 0.07) HBr and 3.63 BBr₃ per mole; then nitric-acid oxidation of the residue brought B to 3.96 (\pm 0.17) per mole. Thus the formula B₄H₈CO seems reliable.

Chemical Behavior.—B₄H₈CO reacts with H₂O or (CH₃)₃N, liberating virtually no carbon monoxide. Further studies are in progress.

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