Table 2. Interatomic distances in $RuAl_6$ and their e.s.d.'s in Å.

Ru—4Al_1	2.628	0.004	Al_2—1Ru	2.492	0.009
2Al_2	2.570	0.009	2Al_1	2.720	0.007
2Al_3	2.618	0.008	2Al_1	2.856	0.008
2Al_3	2.570	0.009	1Al_2	2.587	0.017
			1Al_2	2.679	0.015
Al_1—2Ru	2.628	0.004	2Al_3	2.978	0.011
1Al_1	2.702	0.014	2Al_3	2.903	0.008
2Al_2	2.720	0.007			
2Al_2	2.856	0.008	Al_3—1Ru	2.618	0.008
2Al_3	2.809	0.006	1Ru	2.570	0.009
2Al_3	2.955	0.006	2Al_1	2.809	0.006
			2Al_1	2.955	0.006
			2Al_2	2.978	0.011
			2Al_2	2.903	0.008
			1Al_3	2.739	0.014

distance of 2.57 Å found in $MnAl_6$ has its counterpart in $RuAl_6$ (2.59 Å).

In their work on α-FeCuAl, Black *et al.* showed that this phase was very close in structure to $MnAl_6$, which was originally reported as centrosymmetric.[3] However, they indicated a small departure from centrosymmetry in α-FeCuAl. It was also mentioned in this paper that a similar effect though smaller in magnitude had been observed in $MnAl_6$. Later, an investigation by Walford on $FeAl_6$,[5] which is also of the $MnAl_6$-type, showed that the deviations from centrosymmetry for $FeAl_6$ are less than those in in α-FeCuAl and larger than those in $MnAl_6$.

A test for non centrosymmetry in $RuAl_6$ by further least squares calculations did not give evidence of any departure from the *Cmcm* symmetry. The rather high *B*-value of 2.02 Å^2 for Al_2 in $RuAl_6$ (*cf.* Table 1) might, however, possibly be associated with a slight deviation from this space group. The *B*-values of aluminium found in similar compounds and calculations in, *e.g.*, Rh_2Al_5,[6] Ru_6Al_{13},[7] and Rh_2Al_9[8] are smaller and in the range 0.5—1.8 Å^2. If, however, a deviation from centrosymmetry is present in $RuAl_6$, which cannot be judged from the present experimental data, it is small and certainly less than in α-FeCuAl and in $FeAl_6$.

For valuable discussions and a continuous interest in this work the author is much indebted to professor Arne Magnéli. The investigation has been made possible through the support of the *Swedish Natural Science Research Council* and a scholarship from the *Th. Nordströms testamentsfond* of the *Royal Academy of Science.*

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Biosynthesis of Putrescine: Characterization of Ornithine Decarboxylase from Regenerating Rat Liver

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The biosynthetic routes of spermidine [1] and putrescine [2,3] in bacteria are fairly well known. Working with animal tissues, we have shown that putrescine and methionine serve as precurcors of spermidine and spermine in animal tissues *in vivo*.[4–6] Putrescine is a normal constituent of rat liver and is formed from ornithine *in vivo*.[7] We reported recently that the synthesis of putrescine is markedly increased in the regenerating rat liver.[7] In good agreement with this observation *in vivo*, an increase as high as fortyfold in the ornithine decarboxylase (E.C. 4.1.1.17.) activity, catalysing the stoichiometric formation of putrescine and carbon dioxide, was found in the supernatant fraction from the regenerating liver.[8] In the present work, the latter source has been used for partial characterization of liver ornithine decarboxylase. The crude ornithine decarboxylase obtained by ammonium sulphate fractionation had a pH optimum slightly on the alkaline side and an absolute requirement of pyridoxal phosphate.

Material and methods. The following radioactive compounds, all purchased from the

New England Nuclear Corporation, were used: L-ornithine-U-^{14}C, specific activity 172 mC/mmole, DL-ornithine-1-^{14}C, sp. act. 2.3 mC/mmole, and L-arginine-U-^{14}C, sp. act. 257 mC/mmole. The specific activities given in the text refer to the L-isomer.

The animals used were Wistar rats of both sexes, aged 5 to 8 weeks. For 12 h before sacrifice they were supplied with water only. Partial hepatectomy was performed by the method of Higgins and Anderson [9] 11 to 13 h before sacrifice.

After perfusion, the livers were homogenized with two volumes of cold 0.25 M sucrose solution containing 1 mM mercaptoethanol and 0.5 mM EDTA, pH 7.0, in a Potter-Elvehjem type homogenizer. All subsequent operations were performed below 4°C. The homogenate was centrifuged at 10 000 *g* for 10 min. The supernatant was further centrifuged at 100 000 *g* for 90 min. The resulting supernatant was fractionated with ammonium sulphate (pH 7,0. saturated at room temperature). The precipitates were dissolved in a small volume of 50 mM KCl, pH 7.0, containing 1 mM mercaptoethanol and 0.5 mM EDTA, and dialysed against 4 litres of the same solution for 12 to 16 h. The dialysed sample was brought to 5 mM with mercaptoethanol and stored in a deep-freeze until used. No appreciable loss of the enzyme activity was observed during several weeks' storage. In most experiments the fraction precipitated by ammonium sulphate at 20—45 % was used, since this contained most of the ornithine decarboxylase activity.

Ornithine decarboxylase activity was assayed by measuring the release of $^{14}CO_2$ from DL-ornithine-1-^{14}C. The standard incubation mixture contained 100 μmoles of glycylglycine, pH 7.5, 1 μmole of EDTA, 10 μmoles of mercaptoethanol, 0.5 μmole of pyridoxal phosphate, 2 μmoles of L-ornithine-1-^{14}C (sp. act. 0.05 mC/mmole) and about 5 mg of enzyme protein, in a final volume of 1.0 ml. The enzyme activity is expressed as μμmoles of $^{14}CO_2$ released per mg of protein and per 60 min. The $^{14}CO_2$ was trapped in hyamine and the radioactivity assayed as described earlier.[8] When the formation of ^{14}C-putrescine was determined simultaneously, L-ornithine-U-^{14}C was used. The ^{14}C-putrescine was analysed from the acidified medium as described earlier.[4] Protein was determined by the method of Lowry *et al.*[10]

Results. Although the formation of putrescine could be demonstrated by incubating labelled ornithine with rat liver homogenates, there was no stoichiometric relation between the production of putrescine and CO_2, owing to oxidative metabolism of ornithine. However, most of the putrescine-synthesizing capacity of the homogenate was found in the high-speed supernatant, which catalysed the stoichiometric formation of putrescine and CO_2. Ammonium sulphate fractionation (20—45 % saturation) increased by about threefold the specific activity of ornithine decarboxylase compared with the dialysed crude supernatant. Dialysis of the crude supernatant for 12 h resulted in loss of about 30 % of the activity.

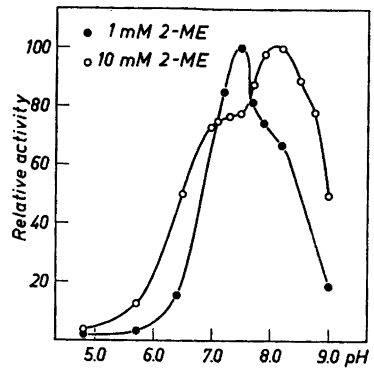

Fig. 1. Effect of pH at two different concentrations of mercaptoethanol (2-ME) on ornithine decarboxylase activity.

The pH optimum of the ornithine decarboxylase activity in the ammonium sulphate fraction was dependent on the concentration of mercaptoethanol in the reaction mixture (Fig. 1). In the presence of 1 mM mercaptoethanol the pH optimum was about 7.4. Increasing the concentration of mercaptoethanol caused a considerable stimulation of ornithine decarboxylase activity, as shown in Table 1, and also

Table 1. Effect of mercaptoethanol on ornithine decarboxylase activity. The standard assay system was used, except that the concentration of mercaptoethanol was varied as indicated.

Mercaptoethanol mM	Ornithine decarboxylase activity
0.1	681
1.0	980
5.0	1 817
10.0	2 244
20.0	2 651

shifted the pH optimum to about 8.1 (Fig. 1). The slope of the curve may indicate the presence of two different enzyme activities, although the separation of these activities by ammonium sulphate fractionation has been unsuccessful so far. Shortage of the starting material as well as the instability of the enzyme (*cf.* above) have hindered further purification.

The dialysed enzyme preparation showed an absolute pyridoxal phosphate requirement. With different concentrations of pyridoxal phosphate in the incubation mixture, 0, 0.2, and 1.0 mM, the production of $^{14}CO_2$ was 73, 969, and 1170 $\mu\mu$moles, respectively. The enzyme was inhibited by hydrazides (Table 2), an observation further supporting the view that pyridoxal phosphate is an essential cofactor. The activation of the enzyme by mercaptoethanol, as well as the inhibition caused by *N*-ethylmaleimide (Table 2), suggest the importance of SH groups for ornithine decarboxylase activity. Neither Mg^{2+} at concentrations from 0 to 20 mM nor EDTA up to 5 mM had any effect on the enzyme activity.

With L-ornithine as substrate, there was a linear relationship between the reaction rate and the concentration of the enzyme. A linear reaction rate was obtained up to at least 60 min. An apparent K_m value of 0.2 mM was obtained for L-ornithine (Fig. 2). Our results also indicate that only the L-isomer serves as substrate, since a decrease of the same magnitude in the production of $^{14}CO_2$ was obtained by diluting the radioactive substrate with 2 mM L-ornithine or 4 mM DL-ornithine. Putrescine even at a concentration of 20 mM caused only a slight inhibition (about 20 %). No effect was produced by adding agmatine up to 20 mM or spermidine up to 10 mM.

Table 2. Effect of some inhibitors on ornithine decarboxylase activity. The reaction mixture *A:* the standard assay mixture containing 10 mM mercaptoethanol; *B.*: as *A.*, but the concentration of mercaptoethanol was reduced to 1 mM.

Reaction mixture	Inhibitor	Concentration mM	Ornithine decarboxylase activity
A.	None		1 182
	Semicarbazide	1	387
		10	88
	Isoniazid	5	572
		10	418
B.	None		551
	N-Ethylmaleimide	1	19

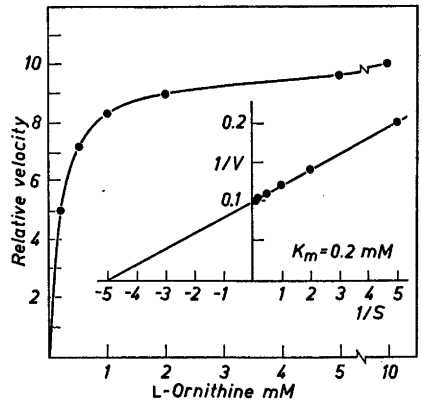

Fig. 2. Effect of substrate concentration on ornithine decarboxylase activity.

Our data also indicate that ornithine decarboxylase is different from other animal amino acid decarboxylases. No inhibition of ornithine decarboxylase activity was observed on adding L-histidine, L-tryptophan, L-lysine, or L-glutamic acid up to 10 mM concentration or L-tyrosine (poorly soluble) up to the saturation concentration. We have also observed (unpublished results) that the activity of L-glutamic acid decarboxylase decreases and that of L-tyrosine decarboxylase only slightly increases after partial hepatectomy.

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A Note on the Reaction of 4,5-Dichloro-1,3-benzenedisulfonamide with Hydrazine and Amines

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The reaction of 4,5-dichloro-1,3-benzenedisulfonamide (I) (Diclofenamide, Daramide®) with hydrazine is reported [1] to result in 6-chloro-3,5-disulfamyl-phenylhydrazine (II) since after removal of the hydrazino-group with alkaline hypochlorite by the method of Chattaway [2] a chlorobenzene-disulfonamide with m.p. 214—215° * was isolated and suggested to be III. The chlorine atom in *o*-, *p*-position to the sulfonamide groups was thought to be sterically hindered.[3]

In connection with studies on benzenesulfonamide-diuretics we have reinvestigated the above sequence of reactions. The obtained chloro-benzenedisulfonamide had m.p. 223—224° * and was identified as 5-chloro-1,3-benzenedisulfonamide (IV) by means of its NMR spectrum (Table 1) indicating the originally formed phenylhydrazine to be V.

The previously suggested structure II of the chloro-disulfamylphenylhydrazine IV has served as a model for the reaction products of I with several amines.[3,5] For this reason we have reinvestigated *N*-carbethoxypiperazino-chlorobenzenedisulfonamide, prepared [5] from I and dechlorinated to 4-(*N*-carbethoxypiperazino)-1,3-benzenedisulfonamide (VI) by hydrogenation. The structure of VI is proved by its NMR spectrum (Table 1).

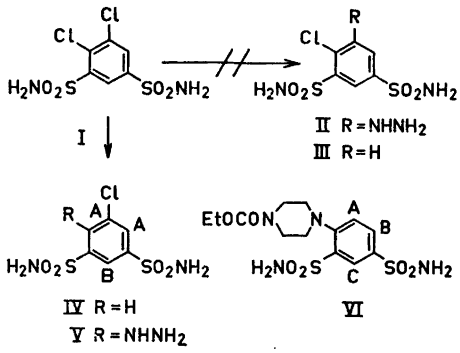

Table 1. NMR data[a] (δ) of IV and VI.

IV[b]		
A	8.09	(d, $J_{A,B}$ = 1.5 cps)
B	8.33	(t, $J_{A,B}$ = 1.5 cps)
VI[c]		
A	8.15	(d, $J_{A,B}$ = 8.6 cps)
B	8.54	(d, $J_{A,B}$ = 8.6 cps; d, $J_{B,C}$ = 2.0 cps)
C	8.91	(d, $J_{B,C}$ = 2.0 cps)

[a] Varian A 60 A (60 MHz);
[b] 10 % in methanol;
[c] 10 % in CF_3COOD, arom. protons recorded only.

The results indicate that the "activated" chlorine atom in the 4-position of I reacts normally with hydrazine and amines and that the earlier reported structure of the reaction products has to be revised in this way.

Experimental. Technical assistance by T. Parbst; Analyses by G. Cornali and W. Egger; NMR spectra by Karin Dehn.

5-Chloro-4-(N-carbethoxypiperazino)-1,3-benzenedisulfonamide. The compound was prepared from 4,5-dichloro-1,3-benzenedisulfonamide and *N*-carbethoxypiperazine as described [5] and purified by several recrystallizations from aqueous ethanol; m.p. 286° (decomp.). (Found: C 36.51; H 4.60; N 12.96. Calc. for $C_{13}H_{19}ClN_4O_6S_2$ (426.89): C 36.57; H 4.49; N 13.12).

4-(N-Carbethoxypiperazino)-1,3-benzenedisulfonamide (VI). To a solution of 1 g of 5-chloro-4-(*N*-carbethoxypiperazino)-1,3-benzenedisulfonamide in 2.7 ml of 2.6 N NaOH and

* 4-Chloro-1,3-benzenedisulfonamide, Lit.[4] m.p. 217—219°; 5-chloro-1,3-benzenedisulfonamide, Lit.[4] m.p. 223—224°.