The enzyme was prepared from *Cl. acetobutylicum* (NRRL-B-527) obtained from the U. S. Department of Agriculture at Peoria, Illinois, and was purified according to Hamilton.[8] (This purified but uncrystallized enzyme is similar to that obtained earlier[2,3] from A.T.C.C. 862, which is unfortunately no longer available.) When the enzyme (100 μg./ml.) in 0.1 M potassium phosphate-citrate buffer at pH 7 was treated with 0.025 M sodium borohydride plus 0.025 M acetoacetate at 0° for 30 minutes, enzymatic activity was irreversibly lost. Enzymatic activity was assayed after exhaustive dialysis by observing the disappearance of the enol absorption band of 0.027 M acetoacetate at 270 mμ in 0.1 M potassium phosphate buffer, pH 5.9 at 30°. The data are recorded in Table I.

TABLE I

Sample, treatment prior to assay	% activity after dialysis
Enzyme	100
Enzyme + 0.025 M acetoacetate	100
Enzyme + 0.025 M BH_4^-	90 ± 5
Enzyme + 0.025 M acetoacetate + 0.025 M BH_4^-	25 ± 5
Same second treatment with acetoacetate plus BH_4^-	12
Same third treatment with acetoacetate plus BH_4^-	7
Enzyme + 0.025 M acetoacetate + 2.5×10^{-4} M HCN	84
Enzyme + 0.025 M acetoacetate + 0.025 M BH_4^- + 2.5×10^{-4} M HCN	69
Enzyme + 0.025 M acetoacetate + 0.025 M BH_4^- + 5×10^{-4} M acetopyruvate	68

Both HCN[9] and acetopyruvate[9,10] are strong, reversible inhibitors of the enzyme. The details of the inhibition by acetopyruvate remain unknown,[10] but HCN may be presumed to add to the C=N bond of a Schiff base intermediate, in the manner of the addition of HCN in the Strecker synthesis. This interpretation is supported by the observation that HCN inhibition develops with perceptible slowness (*e.g.*, 1 min. at pH 6) and that the time lag cannot be shortened by preincubation of HCN with either enzyme or acetoacetate; the inhibition reaction occurs only in the simultaneous presence of enzyme, substrate and HCN. Both HCN and acetopyruvate partially protect the enzyme against reduction and irreversible inhibition by borohydride plus acetoacetate.

The reduction has been performed with 3-C^{14}-acetoacetate (New England Nuclear Corporation). Enzyme (520 μg.) dissolved in 6 ml. of 0.10 M potassium phosphate-citrate buffer, pH 7, was treated with 0.00445 M 3-C^{14}-acetoacetate (1.68×10^6 disintegration/min. μmole) and 0.025 M BH_4^-. After 30 minutes at 0° the solutions were exhaustively dialyzed at 0° against 0.05 M potassium phosphate, pH 5.9, lyophilized and counted with a Packard Model 1314X Tri-Carb Scintillation Counter. The results are shown in Table II.

TABLE II

Sample	D.P.M./200 μg. enzyme
Enzyme + labelled acetoacetate	345
Enzyme + labelled acetoacetate + BH_4^-	5450

On the assumption that the borohydride reduction was 75% complete, the equivalent weight of the enzyme is about 50,000.

A sample of the C^{14} labelled enzyme was hydrolyzed for 24 hours at 110° in 6 N hydrochloric acid in an evacuated, sealed tube and 200 μg. of the hydrolysate subjected to two dimensional paper chromatography.[11] A radioautograph showed a single spot, well removed from both the origin and the solvent fronts.

These results indicate that borohydride reduces a compound formed between the enzyme and acetoacetate, and that this reduction forms a bond stable to acid hydrolysis. The available evidence thus supports the validity of the hypothesis[3] that the enzymatic decarboxylation of acetoacetate, like the amine catalyzed non-enzymatic decarboxylation,[12] proceeds by way of a Schiff base (or Schiff base salt) intermediate. The identification of the group on the enzyme which is reponsible for the formation of the intermediate is actively under investigation.

[8] G. Hamilton, Thesis, Harvard University, 1959.

[9] R. Davies, *Biochem. J.*, **36**, 582 (1942); **37**, 230 (1943).

[10] R. Coleman, Thesis, Radcliffe College, 1962.

[11] R. R. Redfield, *Biochem. et Biophys. Acta*, **10**, 344 (1953).

[12] K. J. Pederson, *J. Phys. Chem.*, **38**, 559 (1934).

THE JAMES BRYANT CONANT LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS
IRWIN FRIDOVICH
F. H. WESTHEIMER

RECEIVED JULY 5, 1962

THE HIGH TEMPERATURE CONTAINMENT OF SUBSTANCES ABOVE THE MELTING POINT OF THE CONTAINER[1]

Sir:

Present high temperature research is close to the practical limit of solid refractory containers set by their melting points. The highest melting metal is tungsten, melting point, 3643°K., the highest oxide is ThO_2, melting point, 3300°K., and the highest melting carbide is TaC, with a melting point 4200°K. A special case is graphite, with a sublimation point of 3800°K.

In practice at our laboratories, these maxima usually cannot be reached due to (1) chemical reaction between container and substance (2) eutectic mixtures, which lower the melting point and (3) thermal shock.

While the limit of thermal stability of chemical compounds is about 5000°K., it was demonstrated[2,3,4] recently that the *liquid range* (*i.e.*, the range from their melting point to their critical point) of some metals extends to 20,000°K. As elementary substances, they cannot decompose, in contrast to chemical compounds. Thus in the case of many liquid metals, experiments could be carried out far

[1] This work was supported by the National Science Foundation under Grant No. NSF-G 18829.

[2] A. V. Grosse, R. I. T. U. Report of Sept. 5, 1960; Paper No. 2159, A.R.S.'s Space Flight Report to the Nation, N. Y. City, Oct. 9-15, 1961.

[3] A. V. Grosse, *J. Inorg. Nucl. Chem.*, **22**, 23 (1961).

[4] A. V. Grosse, *Inorg. Chem.*, **1**, 436 (1962).

beyond the range of the existence of chemical compounds, *i.e.*, $\simeq$ 5000°K.

The problem arises, can substances be contained at high temperatures in liquid containers? This problem was solved[5,6] in principle in 1950, when liquid aluminum metal was boiled, at $\simeq$ 2700°K., in a container of liquid alumina (melting point 2320°K.). A centrifugal chemical reactor was used and it consisted essentially of a large steel pipe, lined with Al_2O_3 bricks, which was rotated horizontally at a few hundred r.p.m. In the free cylindrical volume aluminum metal was combusted in O_2 to Al_2O_3, at an average temperature of 3800°K. The prerequisite for successful operation of a centrifugal reactor is that the density of the liquid reactant *be less* than that of the liquid container.

A limiting disadvantage of a chemical centrifugal furnace is that it must be coupled to an exothermic chemical reaction. Thus no other chemical reactions can be studied except the one actually taking place.

It occurred to us to utilize a high temperature plasma jet[7,8,9] with a noble gas such as helium or argon in the temperature range of 5000 to 15,000°K. as a source of heat for a centrifugal furnace. Such a centrifugal plasma jet furnace now has been operated successfully.

The centrifugal furnace consists of a steel cylinder approximately 12 cm. in diameter surrounded by a water jacket. The unit is rotated in ball bearings by a one horsepower variable drive unit. The r.p.m. range of this unit extends from 500 to 1500. The interior of the steel pipe can be filled with any desired insulating material such as alumina bubbles or Thermofax carbon. The reaction section of the furnace consists of a number of coaxial tubes of any oxide or graphite, depending upon the substance to be contained.

Since the inside diameter of the inner containing tube can vary from 1 to 2.5 cm., a fairly wide range of speeds is necessary to rim[10] the contained material.

The power consumed operating this type furnace varied from 8 to 15 k.w.; voltage was approximately 25 to 30; amperage was approximately 350 to 500. The helium flow was 15 to 30 liters (at N.T.P.)/min. The average temperature of the plasma was 10,000 to 17,500 °K.

An example of the use of the furnace (at 1 atm.) is given. An Al_2O_3 tube was first melted, by heating it for about 5 minutes in the plasma jet; the liquid Al_2O_3 could be observed readily through the exit port, by means of dark glasses. A solid rod of aluminum of known weight was then introduced through the exit port, at a slight angle; it melted in a few seconds and floated on the liquid Al_2O_3 container and came to a boil in about 3 minutes, distilling out through the exit port and burning in air with the usual brilliant flame.

The density of liquid Al_2O_3[11] is 3.053 g./cm.^{3} at the melting point (2288°K.) and 2.569 at 2720°K.; the density of (Al)liq. can be estimated, as has been done for Mg,[12] to be equal to 2.050 at 2720°K., *i.e.*, the N.B.P. of Al.

On letting the rotating furnace cool it was found that the innermost Al_2O_3 tube melted over a length $\simeq$ 10 cm. and the remaining aluminum metal formed a sharp cylindrical band, about 3 cm. wide and $\simeq$ 3 mm. thick, ON the Al_2O_3, the two phases being perfectly defined and separate.

A ThO_2 tube also has been melted in the plasma jet.

The extended range of use of liquid oxide containers is, on the average

Al_2O_3	2288°K. (m.p.) to $\simeq$ 3800°K.
ZrO_2	3000°K. (m.p.) to $\simeq$ 4600°K.
ThO_2	3300°K. (m.p.) to $\simeq$ 4700°K.

The ratio of the vapor pressure of the container to the total pressure can be adjusted, as desired, by operating the plasma jet and furnace at a higher total pressure.

Thus a way is now open to extend research, particularly on chemical reactions in liquid phase, (for example, between the container and any added substance, lighter than the container) to a much higher temperature range. A full report will be published later.

The above method is not well suited for physical measurements (such as density, electrical resistivity, etc.) because of imperfect geometry. This can be accomplished by the use of centrifugal furnaces heated by ohmic resistance. This type of furnace will be described shortly.

---

[5] A. V. Grosse, "High Temperature Symposium Proceedings," June 25–27, 1956, Berkeley, California, pp. 59–68, see Fig. 7 and 8, p. 63.

[6] A. V. Grosse and J. B. Conway, *Ind. Eng. Chem.*, **50**, 663 (1958), see Fig. 5 and 6.

[7] C. S. Stokes, W. W. Knipe and L. A. Streng, *J. Electrochem.*, **107**, 35 (1960).

[8] C. S. Stokes and W. W. Knipe, *Ind. Eng. Chem.*, **52**, 287 (1960).

[9] H. W. Leutner and C. S. Stokes, *ibid.*, **53**, 341 (1961).

[10] R. E. White and T. W. Higgins, *Tappi*, **41**, 71 (1958).

[11] A. D. Kirshenbaum and J. A. Cahill, *J. Inorg. Nucl. Chem.*, **14**, 285–287 (1960).

[12] P. J. McGonigal, A. D. Kirshenbaum and A. V. Grosse, *J. Phys. Chem.*, **66**, 737 (1962).

RESEARCH INSTITUTE OF
TEMPLE UNIVERSITY
PHILADELPHIA 44, PA.

A. V. GROSSE
H. W. LEUTNER
W. J. MURPHY
C. S. STOKES

RECEIVED JUNE 28, 1962

ALKALOID STUDIES. XXXVIII.[1] PILOCEREINE—A TRIMERIC CACTUS ALKALOID[2]

Sir:

With the exception of pilocereine[3] and its isomer, piloceredine,[4] all of the naturally occurring cactus alkaloids[5] are based on a simple β-phenylethylamine or tetrahydroisoquinoline nucleus. Pilocereine had been assigned[3,6] the empirical formula $C_{30}H_{44}N_2O_4$ (496) on the basis of numerous analyses

---

[1] Paper XXXVII. C. Djerassi, R. J. Owellen, J. M. Ferreira and L. D. Antonaccio, *Experientia*, **18**, September (1962).

[2] Supported by grant No. 2G-682 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

[3] G. Heyl, *Arch. Pharm.*, **239**, 451 (1901).

[4] C. Djerassi, T. Nakano and J. M. Bobbitt, *Tetrahedron*, **2**, 58 (1958).

[5] For review see L. Reti in R. H. F. Manske and H. L. Holmes "The Alkaloids," Academic Press, Inc., New York, 1954, Vol. IV, pp. 7–28.

[6] C. Djerassi, S. K. Figdor, J. M. Bobbitt and F. X. Markley, *J. Am. Chem. Soc.*, **79**, 2203 (1957).