have calculated the stoichiometric (1:1) flame temperature (± 50°K.) to increase with pressure as shown:

1 atm.	= 4835°K.
2 atm.	= 4900°K.
5 atm.	= 4985°K.
6.8 atm. (= 100 p.s.i.a.)	= 5015°K.
10 atm.	= 5050°K.

We believe that at the present time these calculated values involve less error possibilities than direct measurements in this 200° range.

To avoid condensation of cyanogen at the higher pressures we have preheated the two gases to 100°. This increased the heat contents of the reactants by 2000 cal./mole and raised our actual flame temperature at 100 p.s.i.a. from 5015°K. to 5050°K. The temperature is not lowered by ionization since all the molecules and atoms involved have very high ionization potentials.

The premixed gases were burned off a torch installed in a pressure chamber.[4] The latter consisted of a 24-inch length of one-inch copper pipe; the top 18 inches of this vertically mounted cylinder were water-cooled. A sight-glass assembly was attached to the chamber just below the water-cooling jacket which allowed for visual observation of the flame during operation. The torch tube was inserted through a stuffing box and positioned in such a way that the flame or torch tip could be seen through the sight-glass. The torch tube was a 10-inch length of stainless steel tubing having an outside diameter of 0.25 inch and an inside diameter of 0.035 inch (= 0.89 mm.). The premixed gas was ignited as it left the tip of the torch by means of a hot tungsten wire igniter. Both gases were measured through Fischer and Porter Flowrators. The reaction chamber was pressurized with argon to the desired value. The combustion products left through a top purge line and a valve was throttled to maintain the desired operating pressure.

The combustion gases were analyzed and found to contain 0.110 mole per cent. CO_2 or less, based on the cyanogen burned. Thus the combustion to CO and N_2 was at least 99.89% complete.

The flame is at its brightest at or very close to the stoichiometric point. It looks like an electric arc even at atmospheric pressure. It becomes more luminous at higher pressures because the combustion takes place in a smaller volume and at a higher temperature.

The way is now open to study the chemistry and physics of various substances in the neighborhood of 5000°K.

Acknowledgment is due to the Office of Naval Research, Department of the Navy (Contract N9-onr-87301) for the financial support of this project, and to the American Cyanamid Company for supplying us with a cylinder of pure cyanogen.

THE RESEARCH INSTITUTE OF TEMPLE UNIVERSITY
PHILADELPHIA, PENNSYLVANIA
J. B. CONWAY
W. F. R. SMITH
W. J. LIDDELL
A. V. GROSSE

RECEIVED FEBRUARY 19, 1955

(4) Details of construction and experimental techniques are fully described in the Final Report on Contract ONR (N9-onr-87301) by J. B. Conway and A. V. Grosse, July 1, 1954, pp. 30–43 and 45.

A NEW PANCREATIC PROTEINASE

Sir:

A previously unreported proteinase has been found in hog pancreas. Its general behavior is not ascribable to trypsin or chymotrypsin. The partially purified material shows considerably stronger hydrolytic activity than crystalline trypsin or α-chymotrypsin upon protein substrates. In Table I a preparation of the new enzyme, temporarily designated pankrin,[1] is compared with crystalline trypsin and α-chymotrypsin in proteolysis of several native (N) and denatured (D) proteins.

TABLE I

COMPARATIVE ENZYMATIC ACTIVITIES[a] AGAINST DIFFERENT SUBSTRATES

Substrate	Trypsin	α-Chymotrypsin	Pankrin	Pankrin / Trypsin	Pankrin / α-Chymotrypsin
D Hemoglobin[b]	11.94	5.70	38.40	3.2	6.7
N Hemoglobin[b]	1.79	1.10	5.36	3.0	4.9
D Serum Albumin[c]	4.77	2.45	6.17	1.3	2.5
N Serum Albumin[c]	0.44	0.38	1.90	4.3	5.0
D Casein[d]	11.52	18.40	31.55	2.7	1.7
N Casein[d]	6.16	9.49	9.54	1.5	1.0

[a] Mg. tyrosine liberated per mg. enzyme in 10 minutes at 37°; Anson, *J. Gen. Physiol.*, **22**, 79 (1938). [b] Bovine hemoglobin substrate powder, Armour. [c] Crystalline bovine serum albumin, Armour. [d] "Vitamin Test" casein, Nutritional Biochemicals.

The markedly high proteolytic activity of pankrin is hard to explain without postulating: (a) a new proteinase; (b) activation of trypsin or α-chymotrypsin; (c) an "unmasking" of additional active centers of trypsin or α-chymotrypsin; or (d) a synergistic action of several proteinases acting at different substrate sites.

A rise in esterase activity of trypsin or α-chymotrypsin, resembling the rise in proteolysis, would be expected to accompany situations (b) and (c). The specific esterase activity of pankrin on *p*-toluenesulfonyl-L-arginine methyl ester (TSAME)[2] is 0.138 and on acetyl-L-tyrosine ethyl ester (ATEE)[3] it is 0.187, but these activities remain well below those of trypsin (TSAME activity = 0.448 and α-chymotrypsin (ATEE) activity = 0.396). Indeed, the ATEE potencies for six fractions of pankrin closely parallel the D hemoglobin potencies (Hb:ATEE = 192 ± 34), suggesting an intrinsic characteristic rather than a chymotrypsin impurity. Possibility (b) is further weakened by retention of activity after dialysis against distilled water and Versene and by the failure of other workers to find any activations of the order of magnitude required here. Possibility (d) fails when tested. Combinations of α-chymotrypsin plus trypsin and/or carboxypeptidase give specific activities (D hemoglobin) lower than that of trypsin. Moreover, pankrin displays vigorous proteinase activity—exceeding that of trypsin—

(1) This name was adopted from the Greek *pankreas*.

(2) G. W. Schwert, H. Neurath, S. Kaufman and J. E. Snoke, *J. Biol. Chem.*, **172**, 221 (1948).

(3) S. Kaufman, H. Neurath and G. W. Schwert, *ibid.*, **177**, 793 (1949).

in the presence of high concentrations of trypsin, chymotrypsin, and carboxypeptidase inhibitors (Table II). Explanation (a) a new proteinase, remains.

TABLE II

INFLUENCE OF INHIBITORS ON PROTEOLYTIC ACTIVITIES

Inhibitor	Trypsin Activity[a]	Trypsin Inhibition, %	α-Chymotrypsin Activity[a]	α-Chymotrypsin Inhibition, %	Pankrin Activity[a]	Pankrin Inhibition, %
None	11.94		5.70		35.80	
Soybean trypsin inhibitor[b]	1.58	87	3.67	36	28.20	21
Pancreatic trypsin inhibitor[b]	2.41	80	4.63	19	24.50	32
β-Phenylpropionate[c] 0.09*M*			1.60	72	16.49	54

[a] Denatured hemoglobin substrate. [b] 100 γ inhibitor per hemoglobin activity unit for each enzyme. This amounts to inhibitor to enzyme weight ratio of 4 (pankrin), 1.2 (trypsin), and 0.6 (α-chymotrypsin). [c] Specific inhibitor for both chymotrypsin and carboxypeptidase: Kaufman and Neurath, *Arch. Biochem.*, **21**, 245 (1949).

Other characteristics of pankrin are these: It is inhibited by DFP and serum. It clots milk. It is unable to clot citrated plasma on any basis of comparison with trypsin—equal weights, proteolytic units, or TSAME units—indicating the probable lack of a trypsin contaminant. Its ATEE splitting activity appears to be less sensitive to β-phenyl propionate than the esterase activity of α-chymotrypsin. Its *p*H optimum (D hemoglobin) is approximately 8.5. Details of purification and substrate specificity will be reported later.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. R. Schlueter and Mrs. G. Cameron.

PROTEIN SECTION
BIOCHEMICAL RESEARCH DEPARTMENT NORMAN H. GRANT
THE ARMOUR LABORATORIES KENNETH C. ROBBINS
CHICAGO 9, ILLINOIS

RECEIVED JANUARY 8, 1955

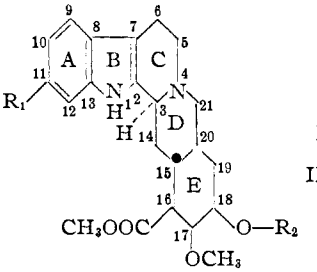
THE STEREOCHEMISTRY OF RESERPINE

Sir:

Recently deserpidine, an alkaloid closely related to reserpine[1] (I), has been isolated from various Rauwolfia species.[2] It was shown to possess structure II and to be stereochemically related to 3-epi-α-yohimbine (which defines the hydrogen atoms at C_{15} and C_{20} to be *trans* to the hydrogen at C_3).

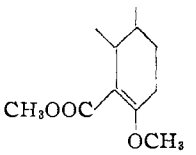
It was furthermore suggested that the same relationship holds for reserpine. We now wish to present chemical evidence for the relative and absolute configurations of four centers (C_{15}, C_{16}, C_{18} and C_{20}) in reserpine.

In the course of our investigation, we had occasion to study the collidine detosylation of methyl reserpate tosylate (III), first reported by Dorfman, *et al.*[1a] By chromatography of the reaction product

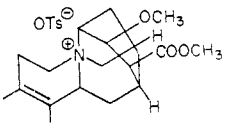


I, R_1 = —OCH_3; R_2 = —$COC_6H_2(OCH_3)_3$
II, R_1 = —H; R_2 = —$COC_6H_2(OCH_3)_3$
III, R_1 = —OCH_3; R_2 = —$O_2SC_7H_7$

on acid-washed alumina we obtained a 10% yield of methyl anhydroreserpate (IV), previously described,[1a] but the major product (30% yield) was a more polar compound which could be eluted with



IV



V

acetone-methanol (20:1). This substance, m.p. 290–291°, $[\alpha]^{23}D$ +88.5° (*c* 0.75, HOAc), was found to be isomeric with methyl reserpate tosylate (III). *Anal.* Calcd. for $C_{30}H_{36}O_7N_2S$: C, 63.36; H, 6.38; N, 4.93; S, 5.63; 3 MeO–, 16.36. Found: C, 63.27; H, 6.23; N, 4.93; S, 5.54; MeO–, 16.16.

That this new tosylate was a quaternary salt was proved by the following observations: It could not be titrated as a base with perchloric acid in acetic acid nor as an acid with sodium hydroxide in 67% dimethylformamide as could the *p*-toluenesulfonic acid salt of methyl reserpate (*pK'*a 7.10). When treated with sodium iodide in acetonitrile it gave an instantaneous precipitate of sodium *p*-toluenesulfonate to form an iodide, m.p. 238–240°, $[\alpha]^{23}D$ +93° (*c* 0.71, MeOH), which was also a neutral salt. *Anal.* Calcd. for $C_{23}H_{29}O_4N_2I \cdot 2H_2O$: C, 49.29; H, 5.94; N, 5.00; I, 22.64. Found: C, 49.99; H, 5.90; N, 4.96; I, 22.31. The infrared spectrum of the isomeric tosylate confirmed its quaternary character since it showed four bands at 8.56, 8.95, 9.71 and 9.94 μ characteristic of the *p*-toluenesulfonate ion[3] but lacked absorption in the 3.8–4.0 μ region indicative of a $>NH^+$ group. From these results structure V was derived for the tosylate salt. Later, it was found that this quaternary compound could also be obtained from methyl reserpate tosylate (III) by refluxing in dimethylformamide. A similar intramolecular displacement reaction has been observed with isorubijervine[3] and other compounds.[4,5]

The formation of an N_4–C_{18} bond requires a *cis* D/E ring juncture, and since reserpine has been shown[2] to have the unstable configuration at C_3, its skeleton must correspond configurationally to that of epi-α-yohimbane. Further, since the quaternary salt (V) is most likely formed by a concerted displacement of tosylate ion by the tertiary nitrogen (N_4) resulting in inversion at C-18, the 18-acyloxy group of reserpine must also be

(1) (a) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. Andre, *Helv. Chim. Acta*, **37**, 59 (1954); (b) C. F. Huebner, H. B. MacPhillamy, A. F. St. Andre and E. Schlittler, THIS JOURNAL, **77**, 472 (1955).

(2) H. B. MacPhillamy, L. Dorfman, C. F. Huebner, E. Schlittler and A. F. St. Andre, *ibid.*, **77**, 1071 (1955).

(3) F. L. Weisenborn and D. Burn, *ibid.*, **75**, 259 (1953).

(4) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(5) B. R. Baker and J. P. Joseph, THIS JOURNAL, **77**, 15 (1955).