TABLE I

PENTAVALENT SALTS OF TRIARYLBISMUTH COMPOUNDS

No.	Compound	Procedure	Recrystn. solvent	Yield, %	M.p., °C. (uncor.) (dec.)	Bismuth, % Calcd.	Found
1	$(C_6H_5)_3Bi(O_2CC_6H_4OH\text{-}o)_2$	2	C_6H_6–petr. ether	52.0	184–185	29.26	29.35, 29.37
2	$(C_6H_5)_3Bi(O_2CC_6H_4OH\text{-}p)_2$[a]	1	Acetone	81.6	250	29.26	29.04
3	$(C_6H_5)_3Bi(O_2CC_6H_4NH_2\text{-}o)_2\cdot C_6H_6$[b]	1	C_6H_6	90.2	95–96	26.51	27.00
4	$(C_6H_5)_3Bi(O_2CC_6H_4NH_2\text{-}p)_2\cdot 2(CH_3)_2CO$	1	Acetone	69.8	148	25.24	25.34
5	$(C_6H_5)_3Bi(O_2CC_6H_4NH_2\text{-}p)_2$[c]	..	.....	..	148	29.33	29.23
6	$(C_6H_5)_3Bi(O_2CCH{=}CHC_6H_5)_2$	2	$CHCl_3$–CH_3OH	50.0	176–178	28.47	28.66
7	$(C_6H_5)_3Bi(O_2CC_6H_4CO_2H\text{-}o)_2$	1	$CHCl_3$–petr. ether	63.3	168–169	27.14	27.00, 27.13
8	$(C_6H_5)_3Bi{-}O_2CC_6H_4CO_2\text{-}o$	2	Aq. alc.	58.3	155–165	34.60	34.87, 34.73
9	$(C_6H_5)_3Bi(O_2CCH_2Cl)_2$	1	Acetone	96.5	155–156	33.33	33.41
10	$(C_6H_5)_3Bi(SC_6H_5)_2$	2	Aq. alc.	35.0	44	31.75	31.44
11	$(p\text{-}CH_3C_6H_4)_3Bi(O_2CC_6H_4OH\text{-}o)_2\cdot C_6H_6$	2	C_6H_6	65.5	164–165	24.99	25.20, 25.52
12	$(p\text{-}CH_3C_6H_4)_3Bi(O_2CC_6H_4OH\text{-}o)_2$	..	.....	..	164–165	27.56	27.49, 27.95
13	$(p\text{-}CH_3C_6H_4)_3Bi(O_2CC_6H_5)_2$	2	$CHCl_3$–CH_3OH	69.0	168–169	28.85	28.66, 29.02
14	$(o\text{-}CH_3C_6H_4)_3Bi(O_2CC_6H_4OH\text{-}o)_2\cdot C_6H_6$[b]	2	C_6H_6	51.0	150–151	25.01	24.99
15	$(p\text{-}ClC_6H_4)_3Bi(O_2CC_6H_4OH\text{-}o)_2$	2	C_6H_6–petr. ether	85.7	187	25.56	26.00, 26.02

[a] Crystallized with acetone of crystallization. Before analysis, the sample was freed of acetone by drying 4 hr. at 95°. [b] Decomposed when attempt was made to free solvent of crystallization. [c] Obtained from solvated compound.

Experimental Part

Procedures 1 and 2 will be illustrated in the examples:

Triphenylbismuth Dianthranilate.—To a boiling solution of 1.0 g. of anthranilic acid in 10.0 ml. of acetone was added 1.3 g. (0.0026 mole) of triphenylbismuth carbonate. Gas was evolved. The mixture was refluxed one-half hour, cooled and diluted with 10.0 ml. of water. The precipitated solid was filtered, dried and recrystallized from benzene.

Tri-*p*-tolylbismuth Disalicylate.—A mixture of 2.1 g. (0.004 mole) of tri-*p*-tolylbismuth dichloride, 1.5 g. of sodium salicylate and 50 ml. of dioxane was shaken 36 hours at room temperature, diluted with 50 cc. of water and the precipitated solid isolated and purified as above.

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The Effect of Competitive Inhibitors on the Milk Clotting Activity of α-Chymotrypsin[1]

BY H. T. HUANG AND CARL NIEMANN[2]

Independent investigations conducted during the past two years[3–5] have provided considerable support for the idea that α-chymotrypsin possesses but one catalytically active site per molecule, and it is now clear that α-chymotrypsin activity can be observed, with selected synthetic substrates, from *p*H 5.5, for L-phenylalanine ethyl ester,[6] to *p*H 8.5, for an acylated-L-tryptophanamide.[7] Furthermore there is evidence[3] that the milk clotting activity of α-chymotrypsin, commonly evaluated at approximately *p*H 5, is associated with the same catalytically active site that is responsible for the hydrolysis of the synthetic specific substrates.

It follows from the above observations that synthetic, low molecular weight, competitive inhibitors of the α-chymotrypsin-catalyzed hydrolysis of synthetic specific substrates, at *p*H 7.9, might be expected to exhibit their inhibitory properties in the α-chymotrypsin-catalyzed milk clotting process if their respective enzyme-inhibitor dissociation constants are not greatly influenced by changes in *p*H. It is clear that the effects associated with *p*H dependencies of modest magnitude can be minimized by evaluating the relative effectiveness of two uncharged and structurally similar competitive inhibitors. Therefore, the inhibitory properties of two such inhibitors of α-chymotrypsin, whose enzyme-inhibitor dissociation constants were previously evaluated at 25° and *p*H 7.9, were tested in respect to their influence upon the milk clotting activity of this enzyme at 25° and *p*H 4.8.

It will be seen from the data given in Table I that the two inhibitors, acetyl-D-tryptophanamide and acetyl-D-tryptophan methyl ester, cause a definite increase in the clotting time over that observed with the control, and while it is doubtful that the results obtained in these experiments are other than qualitative it is of interest to note that the ester is approximately twenty times as active as an inhibitor in the clotting process at *p*H 4.8 than is the amide, a ratio which is roughly the order expected on the basis of their respective K_I values at 25° and *p*H 7.9, *i.e.*, acetyl-D-tryptophan methyl ester, 0.089×10^{-3} *M*[8]; acetyl-D-tryptophanamide, 2.7×10^{-3} *M*,[7] and a similar enzyme-inhibitor dissociation constant *p*H dependency.

TABLE I

INHIBITION OF THE MILK-CLOTTING ACTIVITY OF α-CHYMOTRYPSIN BY TWO REPRESENTATIVE COMPETITIVE INHIBITORS[a]

System	Clotting time in seconds[b] Series A	Series B
No added inhibitor	146 (2.0)	166 (1.7)
With inhibitor I[c]	176 (2.0)	188 (1.5)
With inhibitor II[d]	171 (2.9)	189 (2.6)

[a] Two typical examples from a series of experiments performed at 25° and *p*H 4.8. [b] With indicated standard error based upon a minimum of five separate observations. [c] 2.86×10^{-3} *M* in acetyl-D-tryptophanamide. [d] 0.14×10^{-3} *M* in acetyl-D-tryptophan methyl ester.

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(2) To whom inquiries regarding this article should be addressed.

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Experimental

The assay procedure used was essentially that given by Tauber[9] since it was found that with fresh skimmed milk sharper end-points were obtained than with reconstituted skimmed milk, as recommended by Kunitz.[10] To 90 ml. of fresh skimmed milk at 25° was added 10 ml. of a 0.5 *M* acetic acid–0.5 *M* sodium acetate buffer to give a solution of *p*H 4.8. A mixture of 1 ml. of inhibitor solution, 1 ml. of enzyme solution, containing 0.052 mg. of protein nitrogen per ml., and 5 ml. of buffered milk solution was gently shaken in a thermostat at 25° until clot formation was observed. The inhibitors were those prepared previously[7,8] and the crystalline α-chymotrypsin was an Armour preparation of bovine origin.

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Peculiar Kinetics of Color Formation in Glycerol–Catechol Condensation

BY STEWART C. HARVEY[1] AND VELMA HIGBY

During a study of the kinetics of color development for a microcolorimetric analysis of glycerol[2a] based on the Hovey and Hodgins reaction[2b] a very peculiar stepwise phenomenon was observed. Although this anomaly has been investigated only superficially, it is felt that a note concerning its behavior is warranted.

Experimental

Procedure.—A series of test-tubes were selected for uniformity of diameter and wall thickness in order to ensure equal rates of heat transfer, and the tubes were immersed in an ice-bath after 1 ml. of a solution containing 100 μg. of glycerol per ml. had been added to each. To each was then added 1 ml. of freshly prepared 10% solution of catechol (twice sublimed) following which 4 ml. of a precooled 3:1 solution of sulfuric acid–water were added slowly with gentle agitation in order to avoid excessive premature heating. After a 5 min. period of thermal equilibration the tubes were placed simultaneously in a concentrated sulfuric acid bath of such a capacity and initial temperature that the temperature fell to about 145° when the tubes were immersed in the bath. For the duration of the heating period the temperature was kept at 145 ± 1°. At various intervals tubes were withdrawn, the reaction arrested by immersion in ice-water, and the color intensity read in a Klett–Summerson photoelectric colorimeter or the absorption spectrum determined with a Beckman spectrophotometer.

Results and Discussion

The stepwise character of chromogen formation is shown in Table I in which the results of 10 of 20 such experiments are recorded. Plateau values are shown in bold print and brackets. Over a 10 minute (or more) range two or more steps were observed, each indicating complete cessation of the series of reactions leading to condensation. Patterns determined from different experiments were extremely variable but of similar general character. However, simultaneously withdrawn duplicate and triplicate samples of the same stock generally varied less than 3% in color intensity; furthermore, each plateau value shown represents a separate sample. Therefore, the factors determining the pattern of color development are not randomly distributed among samples of the same solution. In addition, a series of standard glycerol solutions showed color intensities in direct proportion to the concentration,[2a] which suggests that the shape of the curve is also independent of the glycerol concentration. Thus the treppe cannot be explained only as a chain reaction or autocatalysis; the periodic behavior was seemingly determined in some way by fluctuations in the activity of components other than glycerol which were not affected by separation into several tubes. On the other hand, since acrolein reacted to give the chromogen at a much faster rate than did glycerol,[2a] the formation of acrolein from glycerol was the rate-limiting step and rate fluctuations probably involved glycerol transformations. Absorption spectra[2a] of 6, 12 and 18 min. samples varied only in intensity, so that the steps were not a succession of different reactions or polymerizations yielding spectroscopically distinguishable molecular species. When other conditions of glycerol and sulfuric acid concentrations and of temperature obtained, essentially the same type of data was derived.

TABLE I

STEPWISE PHENOMENA IN THE RATE OF COLOR DEVELOPMENT

t, time of heating in minutes; *c*, colorimeter reading with a Klett–Summerson photoelectric colorimeter using a filter of maximum transmission at 540 mμ.

Experiment																					
1		2		3		4		5		6		7		8		9		10			
t	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*	*t*	*c*
1	10	*2*	26	*2*	27	*10*	168	*10*	**179**	*10*	**153**	*10*	142	*10*	137	*11*	**151**	*11*	**174**
2	21	*4*	61	*4*	80	*11*	172	*12*	**179**	*12*	**153**	*13*	156	*13*	179	*11½*	**152**	*11½*	**171**
3	41	*6*	99	*6*	**126**	*12*	**185**	*14*	**179**	*16*	206	*16*	**193**	*16*	200	*12*	**151**	*12*	**185**
4	54	*8*	**132**	*8*	**130**	*13*	**186**	*16*	**208**	*18*	**230**	*19*	**194**	*19*	**248**	*12½*	**151**	*12½*	**185**
5	82	*10*	**132**	*10*	**130**	*14*	**186**	*18*	**210**	*22*	**230**	*22*	228	*22*	**248**	*13*	**170**	*13*	**194**
6	94	*12*	**134**	*12*	**130**	*15*	206	*20*	218	*24*	262	*25*	243	*25*	263	*13½*	**170**	*13½*	**194**
7	119	*14*	197	*14*	206	*16*	229	*22*	233	*28*	**302**	*28*	**277**	*28*	**299**	*14*	**170**	*14*	**194**
8	**152**	*16*	229	*16*	222	*17*	237	*24*	253	*30*	**302**	*31*	**277**	*31*	**304**	*14½*	**183**	*14½*	**194**
9	**154**	*18*	247	*18*	232	*18*	242	*26*	262	*34*	**350**	*34*	315	*34*	**340**	*15*	**183**	*15*	**194**
10	**153**		264	*20*	250	*19*	**253**	*28*	289	*36*	**350**	*37*	350	*37*	**345**	*15½*	202	*15½*	214
						20	**254**	*30*	305	*40*	**397**	*40*	365	*40*	**357**			*16*	**226**
						21	**255**	*32*	354	*42*	**397**	*43*	400	*43*	**357**			*16½*	**224**

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