The structure of the α-carbethoxyindole IV was established by saponification to the acid (m.p. 262°. *Anal.* Found: C, 70.8; H, 4.9; N, 7.3; equiv. wt., 188) and decarboxylation to 1,2-dihydropyrrolo[3,2,1-h,i]indole (VII) (m.p. 76–77°. *Anal.* Found: C, 83.8; H, 6.1; N, 9.6). In each case, the ultraviolet absorption was typical for a substituted indole and practically identical with that of the corresponding compound of the six-membered ring series (VIII).[9]

(9) G. Barger and E. Dyer, THIS JOURNAL, **60**, 2414 (1938).

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RECEIVED JULY 28, 1958

A DIFFERENCE IN BOVINE AND HUMAN FIBRINOPEPTIDES WITH RESPECT TO THE OCCURRENCE OF TYROSINE-O-SULFATE

Sir:

Bettelheim[1] has reported tyrosine-O-sulfate to be a component of fibrinopeptide B, one of the peptides liberated by the action of thrombin on bovine fibrinogen. Recently Blombäck and Vestermark[2] also have reported evidence for the presence of tyrosine-O-sulfate in bovine fibrinopeptide B.

We have confirmed the presence of a component with the properties of tyrosine-O-sulfate in fibrinopeptide of bovine origin but have been unable to detect this compound in peptide derived from human fibrinogen.

Our initial experiments with bovine fibrinopeptide appeared to indicate the presence of a component, readily hydrolyzed by acid, but yielding a product other than tyrosine. The source of these difficulties proved to be the large amount of carbohydrate impurities in clinical thrombin (bovine origin, Parke, Davis and Co.). This material on acid hydrolysis yielded ultraviolet absorption maxima at 283 and 286 mμ in acid and alkaline solution, respectively, which is believed to be due to formation of hydroxymethylfurfural. These reactions either completely obscured or destroyed the liberated tyrosine. Purification of the thrombin according to Rasmussen[3] on Amberlite XE-64 (Rohm and Haas Co.) resulted in removal of interfering materials and a high purity thrombin.[4] Bovine fibrinogen was purified by the procedure of Laki[5] and was 94–96 per cent. clottable. Fibrinopeptide was isolated by the procedure of Bettelheim[6] using purified thrombin. The resulting fibrinopeptide mixture possessed the properties described by Bettelheim[1] with respect to ultraviolet spectra before and after acid hydrolysis. Chromatography of a barium hydroxide hydrolysate of fibrinopeptide on Dowex 1 acetate yielded a peak corresponding in position to that of synthetic tyrosine-O-sulfate.

(1) F. R. Bettelheim, THIS JOURNAL, **76**, 2838 (1954).
(2) B. Blombäck and A. Vestermark, *Arkiv for Kemi*, **12**, 173 (1958).
(3) P. S. Rasmussen, *Biochim. Biophys. Acta*, **16**, 157 (1955).
(4) W. H. Seegers and W. G. Levine, *Seventh Ann. Symposium on Blood*, Wayne State University, Detroit, 1958.
(5) K. Laki, *Arch. Biochem.*, **32**, 317 (1951).
(6) F. R. Bettelheim, *Biochim. Biophys. Acta*, **19**, 121 (1956).

Electrophoresis at *p*H 4.1, using the procedure of Bettelheim,[6] yields two bands with peptide of both bovine and human origin. The mixture of fibrinopeptides isolated from human fibrinogen purified with ammonium sulfate according to Laki[5] or with alcohol according to Morrison, *et al.*,[7] has shown no spectral evidence for tyrosine either before or after mild acid hydrolysis.

Other investigators[8–11] have shown that bovine fibrinogen possesses N-terminal glutamic acid and tyrosine while human fibrinogen possesses N-terminal alanine and tyrosine. The observations reported here suggest that other differences in chemical composition exist in fibrinogen of bovine and human origin.

Acknowledgments.—This investigation has been aided by grants from the Graduate School Research Fund of the University of Minnesota, the Eli Lilly Co. and the Life Insurance Medical Research Fund. Human fraction I was supplied through the courtesy of the American Red Cross and E. R. Squibb and Sons. The assistance of Robert Eric Olson, Robert Kyle and Miss Charlotte Rothnem in preparation of purified thrombin, fibrinogen and synthetic tyrosine-O-sulfate is gratefully acknowledged.

(7) P. R. Morrison, J. T. Edsall and S. G. Miller, THIS JOURNAL, **70**, 3103 (1948).
(8) K. Bailey, F. R. Bettelheim, L. Lorand and W. R. Middlebrook, *Nature*, **167**, 233 (1951).
(9) L. Lorand and W. R. Middlebrook, *Biochem. J.*, **52**, 196 (1952).
(10) L. Lorand and W. R. Middlebrook, *Science*, **118**, 515 (1953).
(11) B. Blombäck, *Arkiv for Kemi*, **12**, 299 (1958).
(12) Fellow of the Helen Hay Whitney Foundation, New York, N. Y., deceased, January 16, 1958.

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RECEIVED AUGUST 12, 1958

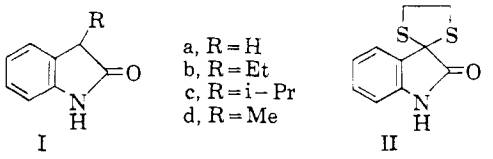
A NEW PROCEDURE FOR FORMING CARBON-CARBON BONDS[1]

Sir:

In connection with our studies in oxindole chemistry[2] we have uncovered a novel method of monoalkylation of active methylene compounds. An investigation of a new synthesis of oxindole (Ia) by a desulfurization of isatin ethylenethioketal (II), m.p. 200–201° (Found: C, 53.92; H, 4.17; N, 6.50) revealed that a Raney nickel treatment of II in benzene or for four hours in ethanol gave the desired product. However, longer runs with W-2 Raney nickel and II, or even Ia, in a 10:1 weight ratio, in various alcoholic solutions yielded 3-alkyloxindoles (I).

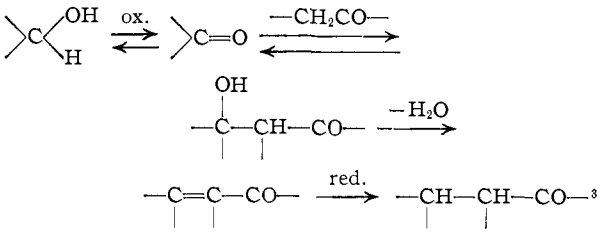
Table I illustrates that (a) oxindole is an intermediate in the conversion of II to Ib-d; (b) a primary alcohol reacts faster than a secondary carbi-

(1) This work was supported by a research grant from the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare (M1301).
(2) *Cf.* E. Wenkert, B. S. Bernstein and J. H. Udelhofen, THIS JOURNAL, **80**, 4899 (1958).



I II

nol; (c) an enhancement of the state of oxidation of the nickel by the introduction of sulfur in form of II or by the addition of *p*-thiocresol (A) or ethylene dithiol (B) to I increases the rate of alkylation. The reaction probably proceeds as shown



The intermediacy of a 3-alkylideneoxindole in the alkylations explains the formation of polymer during methylation.[4]

TABLE I

Reactants	Solvent	Reflux time, hr.	Products and yield, %
II	EtOH	4	63 Ia
II	EtOH	8	21 Ib
II	EtOH	24	83 Ib
II	*i*-PrOH	84	32 Ic
II	MeOH	36	16 Id
Ia	EtOH	72	90 Ib
Ia	EtOH	24	10 Ib
Ia + A	EtOH	24	20 Ib
Ia + B	EtOH	24	50 Ib
Ia	MeOH	70–84	18 Id, polymer

While the C-monoalkylations are novel, their closest analogy is the Raney nickel-induced N-alkylation of primary amines by primary and secondary alcohols.[5] The generality of our method is now under further investigation.

(3) The oxidation-reduction steps find ample analogy in the chemistry of Raney nickel [*cf.* (a) K. Venkataraman, *J. Ind. Chem. Soc.*, **35**, 1 (1958); (b) C. Djerassi, M. Gorman and J. A. Henry, THIS JOURNAL, **77**, 4647 (1955); (c) footnote 18 in E. Wenkert and D. K. Roychaudhuri, *ibid.*, **80**, 1613 (1958)], while the aldol condensation and β-elimination is not surprising in the presence of the very basic catalyst.

(4) For comparable polymer production during a Mannich reaction with oxindole, *cf.* H. Hellmann and E. Renz, *Chem. Ber.*, **84**, 901 (1951).

(5) For discussion of this process, see the interesting review by Venkataraman of his own work and that of others[3a] as well as recent data by C. Ainsworth [THIS JOURNAL, **78**, 1635 (1956)].

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RECEIVED SEPTEMBER 15, 1958

ENZYMATIC REDUCTION OF SULFATE[1]

Sir:

An enzyme system from yeast has been found which catalyzes the reduction of sulfate to sulfite.

(1) Supported in part by the National Science Foundation and the Michigan Agricultural Experiment Station.

The existence of such systems was inferred previously from the ability of fungi and higher plants to utilize sulfate as their sole sulfur source, from studies of nutritional mutants of fungi,[2,3] and from the accumulation of sulfite by plants.[4] Previous workers have reported the oxidation of reduced cytochrome by sulfate in cell suspensions of Desulfovibrio[5] and the formation of cysteine-sulfur from sulfate by acetone-dried *Aspergillus*.[6] *In vitro* reduction of sulfite and thiosulfate, but not sulfate, to hydrogen sulfide has been reported.[7,8]

Sulfite was identified by its volatility after acidification, by the formation of an acid-insoluble barium salt following oxidation with hydrogen peroxide, and by preparation of the crystalline sodium benzoylmethanesulfonate. Following incubation of the enzyme with S^{35}-sulfate and cofactors, the putative $S^{35}O_2$ was released by acidification, purified by passage through $CdCl_2$ in 1% lactic acid, and treated with phenacyl bromide.[9] The specific activity of an infinitely thick sample of the sulfonate became constant at 173 and 168 c.p.m. after one recrystallization; m.p. 257–259° (reported 260°); C, 42.60; H, 3.39; ash, 31.02 (theory: C, 43.24; H, 3.18; ash, 31.96).

TABLE I

Reaction mixture[a]	$S^{35}O_2$ in center well (c.p.m.)	$S^{35}O_2$ formed per vessel[b] (mμmoles)
Complete	2029	14
Minus g-6-p	284	2
Minus ATP	14	0
Minus TPN^+	122	1
Minus Mg^{++}	839	5
Boiled enzyme control	10	0
1.4 mg. enzyme protein	100	1
2.8 mg. enzyme protein	410	3
5.5 mg. enzyme protein	1809	17
11.0 mg. enzyme protein	7830	74

[a] Complete reaction mixture contained in μmoles: g-6-p, 5; ATP, 5; TPN^+, 0.06; $MgCl_2$, 5; K_2HPO_4, *p*H 7, 20; ethylenediaminetetraacetate, 1.4; Na_2SO_3, 5; cysteine, 1.7; $Na_2S^{35}O_4$ (347,000 c.p.m./μmole), 5; dialyzed enzyme protein, 5.5 mg. or as indicated; volume 1.5 ml. Incubation in Warburg vessels for 60 min. at 37°. Reaction stopped with acid and $S^{35}O_2$ collected in center well. Aliquots were analyzed for SO_2 (W. Grant, *Anal. Chem.*, **19**, 345 (1947)) and radioactivity. [b] Corrected for unlabeled SO_2 recovery which ranged from 28 to 53%.

The dependency of the reaction upon the addition of cofactors and upon enzyme concentration is illustrated by the data of Table I. Adenosine triphosphate (ATP), triphosphopyridine nucleotide (TPN^+), glucose-6-phosphate (g-6-p) and enzyme are required. Diphosphopyridine nucleotide and fructose-1,6-diphosphate do not substitute for

(2) D. J. D. Hockenhull, *Biochim. et Biophys. Acta*, **3**, 326 (1949).

(3) N. H. Horowitz in W. D. McElroy and B. Glass, "Amino Acid Metabolism," Johns Hopkins Press, Baltimore, Md., 1955, p. 631.

(4) T. Asahi and N. Harada, *Bull. Agr. Chem. Soc. Japan*, **21**, 243 (1957), and private communication.

(5) J. R. Postgate, *Biochem. J.*, **58**, ix (1954).

(6) C. J. Shepherd, *J. Gen. Microbiol.*, **15**, 29 (1956).

(7) M. Ishimoto, J. Kolyama and Y. Nagai, *J. Biochem. (Tokyo)*, **42**, 41 (1955).

(8) M. Kawakami, T. Iizuka and S. Mitsuhashi, *Japan. J. Exp. Med.*, **27**, 317 (1957).

(9) G. D. Parkes and S. G. Tinsley, *J. Chem. Soc.*, 1861 (1934).