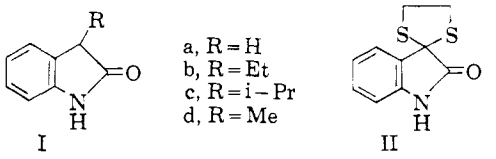
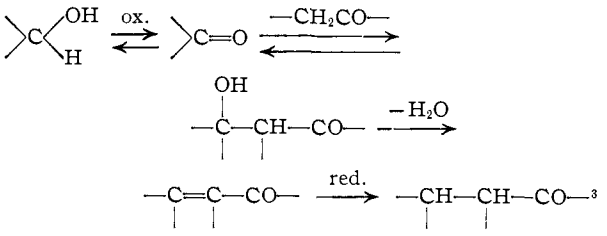


I II

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)].

DEPARTMENT OF CHEMISTRY
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ERNEST WENKERT
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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).

TPN+ plus g-6-p. Magnesium ions are stimulatory. The reaction is a linear function of time over the interval 15 to 120 minutes.

The absolute requirement for ATP suggested that 3'-phosphoadenosine-5'-phosphosulfate (PAPS) might serve as substrate for the sulfate reductase, since previous studies had demonstrated the presence of the sulfate activating system in yeast.[10,11] Incubation of 0.1 μmole of S^{35}-PAPS with the sulfate reducing system (Table I, 1/5th scale) in the absence of ATP yielded 0.5 mμmole of $S^{35}O_2$ although 1.9 mμmoles was formed from an equivalent amount of $S^{35}O_4^=$ (0.1 μmole) and ATP (0.2 μmole). $S^{35}O_2$ was not formed from S^{35}-PAPS in the absence of living enzyme, even with reduced TPN.

(10) R. S. Bandurski, L. G. Wilson and C. L. Squires, THIS JOURNAL, **78**, 6408 (1956).

(11) P. W. Robbins and F. Lipmann, *ibid.*, **78**, 6409 (1956).

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RECEIVED JULY 23, 1958

THE STRUCTURE OF THE DESAURINS

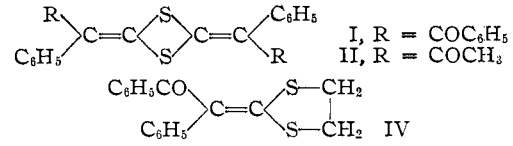
Sir:

The desaurins form a class of stable, high-melting, neutral, yellow compounds obtainable most readily by the reaction of certain ketones with base and carbon disulfide; structure I was suggested for the prototype, the desaurin from deoxybenzoin.[1] Although this proposal subsequently was accepted,[2] it is without experimental support. Such a structure would have considerable interest both in sulfur and in small-ring chemistry. We now report the substantiation of this type of structure for two desaurins.

The desaurin from phenylacetone was obtained as lemon yellow needles, m.p. 233–233.5°, $\lambda_{max}^{CHCl_3}$ 6.05 μ, $\lambda_{max}^{CHCl_3}$ 246 mμ (log ϵ 3.96), 374 mμ (log ϵ 4.44) (*Anal.* Calcd. for $C_{20}H_{16}O_2S_2$: 2 C-CH_3, 8.53; mol. wt., 352. Found: C-CH_3, 7.34; mol. wt., 355).[3] Reduction with zinc and base gave two equivalents of 3-phenyl-2-butanone, establishing the presence of two C_6H_5—C(—C)—C(=O)— residues.

The presence of two carbonyl functions was established by the formation of a *mono*-2,4-dinitrophenylhydrazone, deep red needles, m.p. 250–251°, $\lambda_{max}^{CHCl_3}$ 3.01, 6.05, 6.18, 6.27 μ, $\lambda_{max}^{CHCl_3}$ 356 mμ (log ϵ 4.37), 450 mμ (log ϵ 4.36), a *bis*-2,4-dinitrophenylhydrazone, purple brown powder, m.p. 285–286°, λ_{max}^{KBr} 3.06, 6.19, 6.32 μ, $\lambda_{max}^{CHCl_3}$ 260 mμ (log ϵ 4.26), 341 mμ (log ϵ 4.30), 505 mμ (log ϵ 4.34), and a dioxime, yellow needles, m.p. 209.5–210° dec., λ_{max}^{KBr} 3.00, 6.17 μ, λ_{max}^{EtOH} 250 mμ (shoulder, log ϵ 4.04), 267 mμ (shoulder, log ϵ 3.89), 347 mμ (log ϵ 4.31), 368 mμ (log ϵ 4.29); hydrolysis of the dioxime regenerated the desaurin, indicating the absence of gross structural change on derivativization. The desaurin gave a positive iodoform test and yielded with sodium hypochlorite an acid, $C_{18}H_{12}O_4S_2$, pale yellow microcrystals, m.p. 298–299° dec., λ_{max}^{KBr} 3.0–4.0, 6.02 μ, λ_{max}^{EtOH} 342 mμ (log ϵ 4.50), demonstrating that the carbonyl groups are present as two acetyl groups; this conclusion was corroborated by a strong band at 7.35 μ in the infrared and a single band at 1175 c.p.s.[4] in the n.m.r. spectrum of the desaurin. This evidence in conjunction with the infrared and ultraviolet spectral data requires the presence of two distinct $C_6H_5CCOCH_3$ groupings

and leads unambiguously to the structure II for the desaurin.[5]



The desaurin from deoxybenzoin, golden needles, m.p. 300–302° dec, λ_{max}^{KBr} 6.17 μ, $\lambda_{max}^{CHCl_3}$ 266 mμ (log ϵ 4.36), 419 mμ (log ϵ 4.58), is assigned structure I[5] on the basis of the relationship of its spectral properties to those of II and other evidence given. Oxidation with potassium permanganate or ozone gave benzil and benzoic acid. Reduction with zinc and acetic acid at reflux yielded 1,2-diphenyl-1-propanone (III), 1,2-diphenyl-1-propene and β-1,2-diphenyl-1-propyl acetate; reduction with Raney nickel gave III and β-1,2-diphenyl-1-propanol. The carbonyl stretching band of I is abnormally weak, as is that of IV, yellow needles, m.p. 157.5–158°, $\lambda_{max}^{CHCl_3}$ 6.20 μ, λ_{max}^{EtOH} 257 mμ (log ϵ 4.03), 350 mμ (log ϵ 4.20); this effect is attributed to an *s-cis* conformation of the α,β-unsaturated carbonyl systems. The remarkable stability of the desaurin system is attested by the failure of I to react with concentrated sulfuric acid or with zinc and acetic acid at room temperature, and its recovery (96%) on treatment with concentrated hydrochloric acid at reflux for 100 hours.

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RECEIVED AUGUST 27, 1958

(1) H. Bergreen, *Ber.*, **21**, 337 (1888); V. Meyer, *ibid.*, **21**, 353 (1888); **23**, 1571 (1890); V. Meyer and H. Wege, *ibid.*, **24**, 3535 (1891); W. Wachter, *ibid.*, **25**, 1727 (1892); P. Petrenko-Kritschenko, *ibid.*, **25**, 2239 (1892).

(2) C. Kelber, *ibid.*, **43**, 1252 (1910); C. Kelber and A. Schwarz, *ibid.*, **45**, 137 (1912).

(3) Satisfactory elemental analyses have been obtained for this and all other new compounds here described.

(4) On a scale in which the aromatic toluene peak is assigned a value of 1000 c.p.s.; the spectrum was taken in chloroform solution with a Varian model V4300B spectrometer at 40 mc. rf.

(5) This structure implies no distiuction between the two possible geometrical isomers.