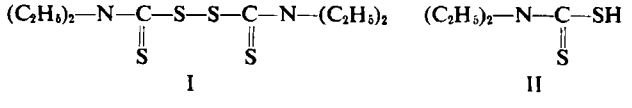
Colorimetric Assay of Disulfiram

RAINER FRIED*▲, ASAAD N. MASOUD†, and FRANCIS M. KLEIN‡

Abstract □ A quantitative assay for disulfiram was developed based on color formation during the interaction of disulfiram, ethanol, and cyanide. A linear curve is obtained over a wide range of disulfiram concentrations. Metabolites of disulfiram do not interfere in this assay. The color reaction is suitable for identification of small aliphatic alcohols and ketones.

Keyphrases □ Disulfiram—colorimetric assay using ethanol and cyanide, used to identify small aliphatic alcohols and ketones □ Alcohols and ketones, aliphatic—identification, colorimetric reaction with disulfiram and cyanide □ Colorimetry—analysis of disulfiram using ethanol and cyanide, used to identify aliphatic alcohols and ketones

During a study of the metabolic effects of disulfiram[1] [bis(diethylthiocarbamoyl) disulfide, I], attention was directed to a color reaction, quoted in the manual of drug identification of Clarke (1). This reaction is found in older manuals and its original discoverer is not known[2]. The color produced by mixing an alcoholic disulfiram solution with aqueous cyanide was listed as a colorimetric spot test for disulfiram. Further investigation showed that this assay could be suitably adapted to a sensitive quantitative method for disulfiram determination. The mechanism and specificity of this reaction were examined further, since the strong blue color, produced rapidly at room temperature, was very puzzling. An examination of a large list of organic compounds proved that the reaction with cyanide and disulfiram could be used as a sensitive class reaction for aliphatic alcohols and ketones and that it may serve as a tool for identification of unknown compounds. Some of the present results were presented as a preliminary communication (2).



I

II

EXPERIMENTAL

Color was developed by mixing a freshly prepared solution of disulfiram (50 mg./5 ml.) in ethanol or other solvents with 1 ml. of 1 *M* NaCN at room temperature. For quantitative determinations, the reaction mixture was let stand for 30 min. at room temperature. TLC was carried out on silica gel GF[3] (3). Disulfiram showed R_f 0.67 in chloroform and R_f 0.24 in benzene, and it could be identified by quenching UV light and by a yellow spot when sprayed with ethanol and cyanide.

Absorption and IR spectra were obtained in spectrophotometers[4]. GC was carried out[5] using stainless steel columns, 1.8 m. × 1.3 cm. (6 ft. × 0.5 in.), with Chromosorb W (100–120 mesh) and 0.5% SE-30 as the liquid phase between 70 and 190°. The reaction mixture with ethanol was investigated further and was tested for diethyl dithiocarbamate (II) and carbon disulfide by colorimetric methods (4, 5). Sulfur was identified as barium sulfate after wet-ashing with concentrated nitric acid, and thiocyanate was identified by reaction with ferric chloride (6).

RESULTS AND DISCUSSION

An ethanolic solution of disulfiram treated with 1 *M* sodium cyanide rapidly forms an intense yellow color, which quickly passes to green and blue, with a broad maximum between 520 and 580 nm. and a minimum at 450–460 nm. (Fig. 1). The blue color fades on standing, reverting to yellow; neither the intensity of colors nor the rate of color formation is affected by the order of addition. The blue color can be extracted into ether but does not pass into petroleum ether.

When the graded levels of disulfiram are used for color development, a straight line is obtained over a wide concentration range. This can be used as a convenient colorimetric assay for disulfiram (Fig. 2). Eventual interference by other colored compounds, as found in urine, can be eliminated by extracting the blue color into ether and determining the color of the organic phase. Diethyl dithiocarbamate (II) and carbon disulfide, important metabolites of disulfiram (7–11), do not give a color reaction with cyanide, nor are they formed in the reaction of disulfiram with cyanide. The present method is, therefore, suitable for studies of metabolic transformations of disulfiram.

Color formation only takes place with oxygenated compounds that act not only as solvents but also participate in the reaction (Table I). Best results are obtained with small primary aliphatic alcohols, of which butanol forms an intense blue color at the interphase. The blue color is most stable and intense when methoxyethanol or *tert*-butanol is used as a reactant. Acetaldehyde could

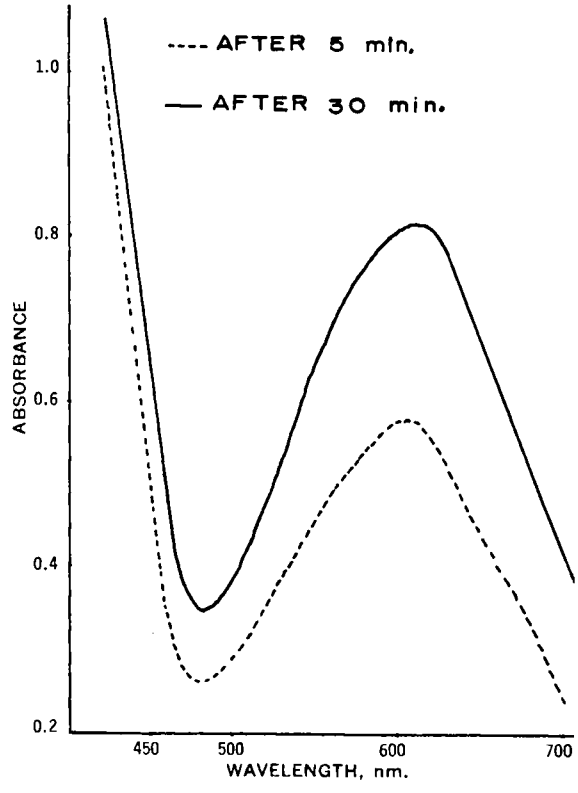


Figure 1—*Absorption spectrum of reaction product of the cyanide–disulfiram ethanol condensation. Key: - - -, after 5 min. at 25°; and ——, after 30 min. Thirty milligrams of disulfiram in 5 ml. of ethanol was used.*

[1] Antabuse.
[2] E. G. C. Clarke, personal communication.
[3] Merck.
[4] Beckman DB and IR-8 spectrophotometers, respectively.
[5] In a Beckman GC 2A.

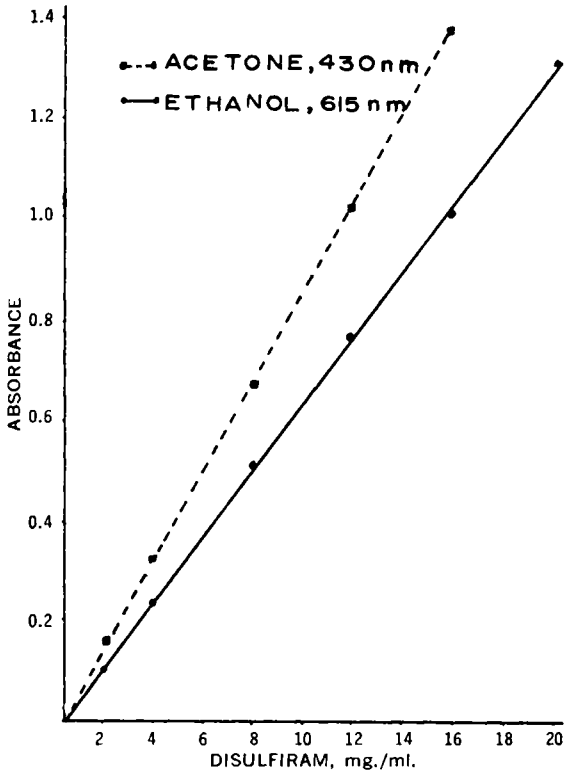


Figure 2—*Standard curve for disulfiram, with graded levels of disulfiram in 5 ml. of solvent, and 1.0 ml. of 1 M NaCN added. Absorbance was read after 30 min. at 25°.*

not be tested, because acetaldehyde and cyanide alone (in absence of disulfiram) form an intense brown color in a strongly exothermic reaction. An intense yellow color is formed by acetone, dioxane, dimethyl sulfoxide, and methylcellulose. Contrary to the reaction with alcohols, this color is stable and does not pass to blue nor fade on standing.

Acetone also serves as a suitable reagent for quantitative determination of disulfiram (Fig. 2). Dimethyl sulfoxide gives an exothermic reaction in which gas and precipitate formation are observed. When the reaction is carried out in this reagent, the yellow color is not converted to blue on addition of ethanol.

To identify the products of this reaction, a large batch was prepared with ethanol using the same proportions of reagents as for the routine color development. Several compounds were isolated, and the presence of others was detected by TLC. The new reaction products were found not to be disulfiram or its known derivatives by using TLC, GC, and UV and IR spectrophotometry. In view of the rapid formation and great intensity of color, a clarification of the reaction mechanism would be of considerable theoretical interest.

A proposed mechanism (12, 13) may be pertinent, in which a disulfide bridge is split by cyanide, with the formation of a thioether and free thiocyanate. However, no color formation has been reported (13). Thiocyanate could not be detected in the present study by reaction with ferric chloride; the reasons for these discrepancies are not clear. Similar interactions of cyanide with disulfide bridges occur not only with simple organic compounds but also with biological compounds. Cyanide causes cleavage of protein chains at the cystine bridges (14, 15) with the liberation of thiocyanate (16). Xanthine oxidase is irreversibly inactivated by cyanide, which forms a covalent bond with the enzyme (17, 18), and thiocyanate is liberated from the persulfide group, which is essential for enzyme activity (19).

The present procedure was used to determine disulfiram in urine of chronic alcoholics receiving disulfiram therapy. The drug could not be identified in urine or urine extracts, which is in agreement with previous reports (4, 20, 21).

SUMMARY

The interaction of disulfiram, cyanide, and organic solvents was studied. A simple colorimetric assay of disulfiram was developed, which gives a linear response over at least a 20-fold range. The reaction can be used as a type reaction for alcohols.

Table I—Color Reaction of Disulfiram with Organic Reagents[a]

Reagent	Color	Reaction Rate[b]
Methanol	Blue	Fast
Ethanol	Blue	Fast
Isopropanol	Green	Fast
1-Butanol	Blue	Fast
Methoxyethanol	Blue	Fast
2-Butanol	Light green	Slow
tert-Butanol	Blue	Fast
Acetaldehyde[c]	(Brown)	(Fast)
1-Octanol	Light orange	Very slow
Isoamyl alcohol	Yellow	Slow
Acetone	Lemon yellow	Fast
Dioxane	Brown	Fast
Formamide	Yellow	Slow
Dimethylformamide	Yellow	Slow
Dimethyl sulfoxide[c]	Yellow	Fast

[a] Fifty milligrams of disulfiram was dissolved in 5.0 ml. of solvent, and 1.0 ml. of 1 *M* NaCN was added. [b] Negative reactions were obtained with cyclohexanol, benzyl alcohol, phenol, acetic acid, ethyl acetate, butyraldehyde, hexane, benzene, ether, chloroform, carbon tetrachloride, and carbon disulfide. [c] See text.

REFERENCES

(1) E. G. C. Clarke, "Isolation and Identification of Drugs," The Pharmaceutical Press, London, England, 1969, p. 319.

(2) R. Fried and A. Masoud, *Fed. Proc.*, **31**, Abstr. 580(1972).

(3) E. Stahl, "Thin-Layer Chromatography," 2nd ed., Springer, New York, N. Y., 1969.

(4) S. L. Tompsett, *Acta Pharmacol. Toxicol.*, **21**, 20(1964).

(5) H. Casier and E. Merlevede, *Arch. Int. Pharmacodyn. Ther.*, **139**, 165(1962).

(6) F. P. Treadwell and W. J. Hall, "Analytical Chemistry," Wiley, New York, N. Y., 1942, I, 215; II, 320.

(7) L. Eldjarn, *Scand. J. Clin. Lab. Invest.*, **2**, 202(1950).

(8) J. H. Strömme, *Biochem. Pharmacol.*, **14**, 393(1965).

(9) R. Fischer and H. Brantner, *Naturwissenschaften*, **50**, 551 (1963).

(10) C. S. Prickett and C. D. Johnston, *Biochim. Biophys. Acta*, **12**, 542(1953).

(11) E. Merlevede and H. Casier, *Arch. Int. Pharmacodyn. Ther.*, **132**, 427(1961).

(12) E. E. Reid, "Organic Chemistry of Bivalent Sulfur," vol. IV, Chemical Publishing Co., New York, N. Y., 1962, p. 250.

(13) A. Cambron, *Can. J. Res.*, **2**, 341(1930); through *Chem. Abstr.*, **25**, 3041(1930).

(14) N. Catsimpoolas and J. L. Wood, *J. Biol. Chem.*, **238**, 2887 (1963).

(15) *Ibid.*, **241**, 1790(1966).

(16) *Ibid.*, **239**, 4132(1964).

(17) M. Dixon and D. Keilin, *Proc. Roy. Soc., Ser. B*, **119**, 159 (1936).

(18) I. Fridovich and P. J. Handler, *J. Biol. Chem.*, **231**, 899 (1958).

(19) V. Massey and D. Edmondson, *ibid.*, **245**, 6595(1970).

(20) J. Hald, E. Jacobsen, and V. Larsen, *Acta Pharmacol. Toxicol.*, **4**, 285(1948).

(21) G. Domar, A. Fredga, and H. Linderhold, *Acta Chem. Scand.* **3**, 1441(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 6, 1972, from the *Department of Biochemistry, School of Medicine, †Department of Biological Science, School of Pharmacy, and ‡Department of Chemistry, College of Arts and Sciences, Creighton University, Omaha, NB 68131

Accepted for publication February 16, 1973.

The authors thank Ayerst, New York, N. Y., for a generous supply of disulfiram, and Mr. Charles Ditch and Dr. Charles Bonniwell (Immanuel Hospital, Omaha, Neb.) for their interest and cooperation.

▲ To whom inquiries should be directed.