Journal of Chromatography, 116 (1976) 230–234

CHROM. 8650

Note

Chromogenic response of aqueous cobalt thiocyanate to lipophilic drugs

FRED W. GRANT

Research Center, Rockland Psychiatric Center, Orangeburg, N.Y. 10962 (U.S.A.)

(First received March 13th, 1975; revised manuscript received July 22nd, 1975)

Two percent aqueous cobalt thiocyanate (CTC) has found widespread application in the analysis of cocaine[1]. The test relies on a change in the color of the reagent from pink to blue in the presence of drug. Its relatively poor specificity for cocaine was brought to our attention by local drug control agencies and led to the development of a superior test not based on CTC[2].

It was of interest to determine if CTC might make useful distinctions among drug types as a spray reagent in drug screening procedures based on thin-layer chromatography (TLC). Two percent CTC in acetone has been reported to distinguish certain alkaloids on the basis of the extent and rate of color development[3]. The advantages of acetone as a solvent were not described nor was a positive test response associated with any particular drug property.

Our earlier efforts in this direction[4] pointed to a unique quality of CTC in responding to the more highly substituted lipophilic homologs of certain drugs. Heroin responds readily to the aqueous reagent, codeine less so, and morphine not at all; dimethyltryptamine responds but not tryptamine, methamphetamine responds weakly but more than amphetamine. We suggested that a ligand-sensitive thiocyanate–isothiocyanate linkage isomerism may be implicated in explaining these results based on analogy with the steric and polar effects operating in related transition metal thiocyanate complexes[5].

It is apparent that CTC is responsive to the lipophilic character of drugs, a property the importance of which in drug transport and action is increasingly evident[6]. We report here our further experience with the nature and scope of this reagent in drug testing.

EXPERIMENTAL

Chromatography

The results reported here were obtained using precoated silica gel F-254 TLC plates (Merck, Darmstadt, G.F.R.). While the choice of adsorbent had little if any effect on the CTC–drug response, background interference was significant with Florisil which develops an immediate blue color indistinguishable from the test color. Adsorbents based on silica gel and, to a lesser extent, alumina will also develop a blue background color as the plate dries. This generally necessitates that test results be interpreted within 5–10 min after spraying. However, background color may be

repeatedly and selectively removed by exposure to a water-saturated atmosphere. Test color or persistence did not change when commercial grades of acidic, basic, and neutral alumina were used as TLC adsorbents.

Test drugs were spotted in graded concentrations on the TLC plates and developed for a solvent front distance of 10 cm with 0.5% ammoniacal methanol. The CTC reagent was insensitive to the acidity, basicity, or any other property of the developing solvent as long as the usual amount of forced air or oven drying preceded the application of the reagent.

Test reagent

A less than saturating application of 2% aqueous cobaltous thiocyanate (K & K Labs., Plainview, N.Y., U.S.A.) was applied to the dried plate with a conventional spray applicator. Since water will ultimately wash out a positive test result, solutions more dilute than 2% were self-defeating and more concentrated solutions served no useful purpose. No serious effort was made in this study to modify the reagent in order to optimize or alter the test result.

That water is an important part of the test reagent is brought out by the use of 2% CTC in acetone. This reagent has been reported useful in making distinctions between certain alkaloids in TLC applications[3]. In our hands, test responses were significantly weaker and less persistent. The blue test and background colors are completely bleached out after standing for several days whereas the colors deriving from the aqeuous reagent are stable indefinitely. No advantages to the use of the acetone reagent were observed.

Test drugs

Test drugs were obtained either in unadulterated form from commercial sources or in prescribed dosage form from the Pharmacy of the Marcy Psychiatric Center (Marcy, N.Y., U.S.A.) Cannabis extract, methaqualone, and diacetylmorphine were obtained from confiscated material which had been submitted to our laboratory for analysis. Dimethoxyphenethylamine (DMPEA) heptanoate was synthesized by unambiguous means from commercial DMPEA and was isolated in crystalline form. Arctigenin was isolated by column chromatography of the chloroform extract of Arctium lappa seeds collected locally.

RESULTS AND DISCUSSION

The drugs tested in this study were arbitrarily divided into hydrogen donor drugs (Table I) and hydrogen acceptor drugs (Table II) by familiar criteria[7]. Drugs with mixed donor–acceptor properties were included in Table I as hydrogen donors. Available measured or calculated octanol–water partition coefficients[7] are listed in Tables I and II as drug parameters which correlate reasonably well with CTC–drug response. The average partition coefficient of those drugs responding to CTC at the level of 20 μg or less CTC (++ and + in Tables I and II) in this study was 3.05 $\pm$ 0.30 (standard error of the mean). The average partition coefficient of those drugs that did not respond to CTC, or whose response required more than 20 μg, was 1.21 $\pm$ 0.20. This difference was significant at the $p < 0.001$ level by t-test, two-tailed.

As lipophilic or hydrophobic character further increases, CTC response di-

TABLE I

RESPONSE OF HYDROGEN ACCEPTOR* DRUGS TO COBALT THIOCYANATE

Response: −+, detectable after TLC development at the level of 10 μg or less; +, 10–20 μg: −, more than 20 μg or undetectable.

Drug	*Response to cobalt thiocyanate*	*Octanol–water partition coefficient**
Amitriptyline	−+	
Amphetamine	−	1.63
Benzphetamine	−+	2.47
Benztropine	−+	
Caffeine	−	−0.07
Chlorpheniramine	+	
Chlorpromazine	−+	5.16
Cocaine	−+	2.73
Cyproheptadine	++	
Dextromethorphan	−	2.91
Diacetylmorphine	+−	
Diazepam	−+	2.82
Dihydrocodeinone	−	
Dimethoxyphenethylamine	−	
Dimethyltryptamine	−+	
Diphenhydramine	−+	3.30
Doxepin	++	
Ethopropazine	−+	
Ethyl aminobenzoate	−	2.41
Imipramine	+−+	4.62
Ladanosine	+	
Lysergide	−	
Meperidine	−	
Mescaline	−	
Methadone	+−+	
Methamphetamine	−	2.12
Methapyrilene	−+	
Methaqualone	−	
Methenamine	−	
Methylphenidate	−	
Nicotine	−	1.17
Nortriptyline	+	
Papaverine	+−	
Phenmetrazine	−	1.73
Phenyltoloxamine	+	
Procaine	−−+	1.87
Promethazine	+	2.25
Propoxyphene	−−+	4.18
Protriptyline	−−	
Strychnine	+	1.93
Tetracaine	−+−	3.73
Thiothixene	+	
Tripelennamine	−−+	
Tryptamine	−	

* As described in ref 7.

TABLE II

RESPONSE OF HYDROGEN DONOR* DRUGS TO COBALT THIOCYANATE

Drug	*Response to cobalt thiocyanate***	*Octanol–water partition coefficient**
Acetaminophen	—	
Acetylsalicylic acid	—	1.23
Arctigenin	++	
Atropine	—	1.79
Bufotenine	—	
Cannabis hexane extract	—	
Chlordiazepoxide	—	2.44
Codeine	—	1.01
Colchicine	++	
Cyclandelate	—	
Dimethoxyphenethylamine heptanoate	++	
Ephedrine	—	0.93
Glutethimide	—	1.90
Haloperidol	+	
Hydromorphone	—	
Hydroxyzine	+	
Isocarboxazid	—	1.49
Lidocaine	+	
Meprobamate	—	0.70
Morphine	—	0.76
Nylidrin	+	
Oxazepam	—	
Pentazocine	+	
Phenobarbital	—	
Physostigmine	—	0.17
Procyclidine	++	
Quinine	+	1.73
Salsoline	—	
Scopolamine	—	
Sulfisoxazole	—	1.15
Theobromine	—	−0.78
Trihexyphenidyl	++	

* As described in ref. 7.
** As defined in Table I.

minishes to a weak, delayed response, such as we have observed in the case of fatty esters such as glyceryl tristearate, and finally to no response in the case of aliphatic or aromatic hydrocarbons. Nitrogen content is not essential since water-miscible solvents such as acetone and methanol give a strong blue test color as does the weakly acidic arctigenin (Fig. 1). Thus, a positive test result entails a rather narrow compromise between hydrophilic and lipophilic character in the substance tested.

It is apparent from this study that CTC–drug response can often be promoted by the synthesis of a suitable derivative. This is dramatically illustrated by the acetylation of morphine and by the N-methylation of tryptamine.

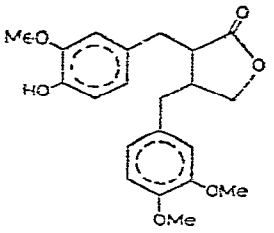

Fig. 1. Arctigenin.

ACKNOWLEDGEMENT

This work was supported by the Office of Research of the New York State Department of Mental Hygiene.

REFERENCES

1 Anonymous, *Drug Enforcement*, 1 (1974) 26.
2 F. W. Grant, W. C. Martin and R. W. Quackenbush, *Bull. Narcotics*, 27 (1975) 33.
3 G. Zweig and J. Sherma, *Handbook of Chromatography*, Vol. 2, Chemical Rubber Company Press, Cleveland, Ohio, 1972. p 112.
4 F. W. Grant and R. W. Quackenbush, *Abstracts of the Sixth Northeast Regional Meeting of the American Chemical Society, Burlington, Vt., August 19, 1974.*
5 W. Beck and W. P. Fehlhammer, *MTP Int. Rev. Sci., Inorg. Chem., Ser. I*, Vol. 2, Butterworths, London, 1972, pp. 267–279.
6 R. N. Smith, C. Hansch and M. M. Ames. *J. Pharm. Sci.*, 64 (1975) 599.
7 A. Leo, C. Hansch and D. Elkins, *Chem. Rev*, 71 (1971) 525.