The Direct Analysis of Tetracycline in Urine by Circular Dichroism Spectropolarimetry

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Received August 4, 1981, from the *Chemistry Department, Oklahoma State University, Stillwater, OK 74078.* Accepted for publication October 23, 1981.

Abstract □ A method is described for the direct analysis of tetracycline in human urine using circular dichroism spectropolarimetry. The general applicability of the method to other drugs is discussed.

Keyphrases □ Circular dichroism—spectropolarimetry, tetracycline analysis in urine □ Tetracycline—analysis in urine, circular dichroism spectropolarimetry □ Spectropolarimetry—tetracycline analysis in urine, circular dichroism

Circular dichroism is the effect caused by the simultaneous absorption and rotation of an incident beam of plane polarized light measured as a function of wavelength (1). Ordinarily the technique is used in the study of electronic transition polarizations and in the investigation of molecular conformations or configurations (2).

In previous work its analytical potential was demonstrated in the qualitative analysis (3, 4) and quantitative determination of drugs of abuse, such as opiates (5), L-cocaine (6), and lysergide (7). The analyses were made directly on confiscated solid samples of forensic interest, meaning no prior sample preparation or separation was performed, nor was it necessary.

This report describes the preliminary results from the first application of circular dichroic spectropolarimetry to the direct analysis of drugs in biological fluids, namely tetracycline in human urine.

EXPERIMENTAL

An analytical standard sample of tetracycline hydrochloride was obtained[1]. Urine samples were taken from 10 volunteers who were known not to be on a program of administering drugs, legal or illegal. One volunteer was maintaining an established program of a 1.0-g daily dosage of tetracycline[2] using 500-mg capsules.

Circular dichroic spectra were taken on a spectropolarimeter[3] over the 240–360-nm wavelength range. Measurements were made on freshly collected specimens which had been diluted 50 times with distilled water. The pH of the solution was not controlled. Samples were placed in a 1-cm cell and measurements were made against a distilled water blank.

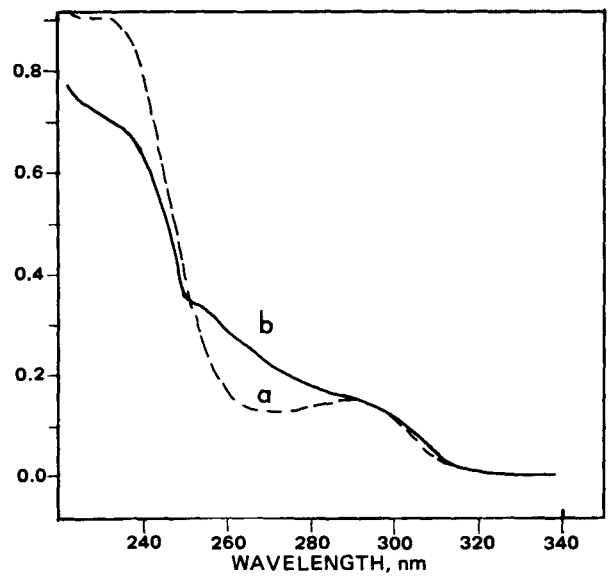

Figure 1—*UV absorption spectra of (a) urine (50 times dilution) and (b) urine with tetracycline (50 times dilution).*

RESULTS

The UV absorption spectra of urine with and without tetracycline from the prescribed volunteers are shown in Fig. 1. The principal difference in absorption, ~270 nm, is insufficient to quantitate with any degree of certainty. Even qualitative identification is speculative. Circular dichroic spectra of the same specimens are shown in Fig. 2. Samples had been diluted 50 times to prepare these solutions. The observed circular dichroic spectrum for urine shows small deviations from the baseline: negative at ~310 nm and positive at ~280 nm. Tetracycline in urine shows a large positive Cotton band at 295 nm and two smaller negative bands at 323 and 270 nm, respectively. Superimposed on the urine spectra of Fig. 2 is the spectrum for a 2.0×10^{-5} *M* or 8.9 μg/ml aqueous tetracycline solution. Positive qualitative identification is elementary.

The quantitativeness of circular dichroic spectropolarimetry in analysis can be demonstrated from a linear plot of experimental ellipticity, Ψ, at 295 nm *versus* molar concentration of standard tetracycline[1] in water. The slope of the calibration curve is the molar ellipticity coefficient $[\theta]_{295}$ and is calculated to be equal to +540. Using this value in the analysis of the urine samples, the concentration of dissolved tetracycline was calculated to be 2.4×10^{-5} *M* after dilution by 50 times, or 11 μg/ml in the excreted specimen.

With the present instrument, the limit of detection was found to be ~1.8 μg/ml for tetracycline.

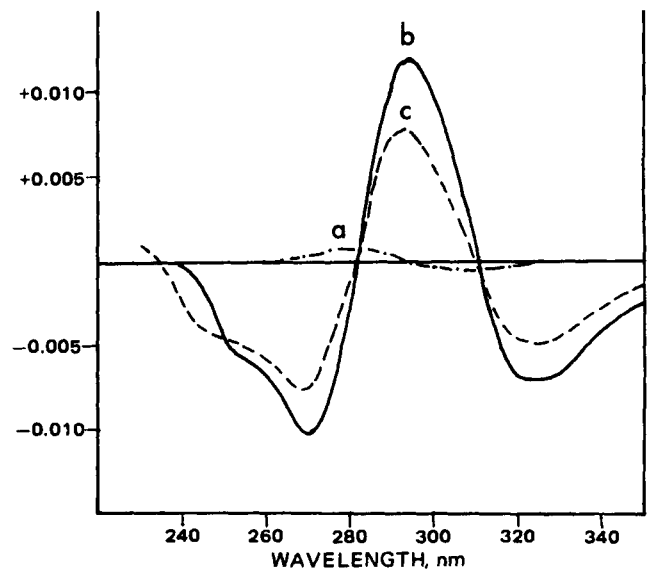

Figure 2—*Circular dichroic spectra of (a) urine (50 times dilution), (b) urine with tetracycline (50 times dilution), and (c) 2×10^{-5} M aqueous solution of tetracycline.*

[1] From the Drug Enforcement Administration *via* the Oklahoma State Bureau of Investigation.

[2] Panmycin, Upjohn Chemical Co.

[3] Cary 61, Varian Instruments, Inc.

0022-3549/82/0700-0836$01.00/0

DISCUSSION

Circular dichroic spectropolarimetry is a modified form of UV absorption spectrophotometry applicable to compounds which are both optically active and absorb light (2). All of the accepted procedures for UV absorption apply equally well to circular dichroism, and the data obey the simple Beer's Law dependence. Adjusting instrument parameters is not a problem as it is with GC, high-pressure liquid chromatography, or mass spectrometry (8) which reduces the routine analysis time. The time is reduced even further where separation is not a prerequisite to identification.

The detection limit for tetracycline in urine is 1.8 μg/ml. This value easily could be improved with a more modern instrument equipped with computer data handling accessories. A lower limit of detection is also possible if tetracycline is first separated from other species which absorb in the UV because of an improved signal–noise ratio. Based upon known $[\theta]$ values for other drugs such as morphine (5), codeine (5), cocaine (6), and lysergide (7), and comparing these to the value for tetracycline, calculations show that these drugs also can be quantitated, but only at overdose levels with the spectropolarimeter[3].

In general terms the most difficult analytical problem will arise when a mixture of optically active drugs are present (7); then separation will again be necessary. Dissolved sugars or proteins and glucuronide derivatives of extracted metabolites do not absorb for the most part, and they are not interfering.

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ACKNOWLEDGMENTS

This work was supported by NSF-CHE-7909388.

The authors thank Mr. Don Flynt of The Oklahoma State Bureau of Investigation for supplying the standard sample of tetracycline hydrochloride.

Tick Repellents I: Ethylene Glycol Acetamides

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Received July 7, 1981, from the *Life Sciences Division, SRI International, Menlo Park, California 94025.* Accepted for publication September 25, 1981. * Present address: Bayer AG, Postfach 101709, 5600 Wuppteral, West Germany.

Abstract □ Acetamides derived from ethylene glycol were synthesized and evaluated as repellents for the brown dog tick *Rhipicephalus sanguineus*. Several of these compounds showed repellency equal to the standard repellents, *N,N*-diethyl-*m*-toluamide and butopyranoxyl.

Keyphrases □ Tick repellents—ethylene glycol acetamides, synthesized □ Acetamides—ethylene glycol, synthesized, tick repellents □ Ethylene glycol—synthesized acetamides, tick repellents

A previous study of insect repellents showed that alkyl triethylene glycol monoethers had good mosquito repellency, being superior to *N,N*-diethyl-*m*-toluamide in certain tests against *Aedes aegypti* mosquitoes (1). Amides in general are known to be repellent to mosquitoes and other insects, the most widely used amide repellent being *N,N*-diethyl-*m*-toluamide.

During World War II, an extensive repellent screening program took place at the USDA Laboratories in Orlando, Florida. Compounds were screened for repellency both to yellow fever and malaria mosquitoes, and against fleas and ticks (2, 3). The ticks used for that program were the lone star tick, and about one thousand compounds were evaluated as tick repellents.

Since that time, major emphasis has been on mosquito repellents, largely supported by the U.S. Army Medical Research and Development Command. However, recently more emphasis has developed with regard to other militarily important insects: sand flies and ticks.

A previous study (3) evaluated a series of amides against ticks (*Amblyomma americanum*) and found that the di-*n*-butyl toluamides were best. Another study (4) found that certain amides and esters were effective against hard and soft ticks (*Ixodes persulcatus* P. Sch., *Dermacentor pecitus* Herm., *D. marginatus* Salz., *Hyalomma asiaticum* P. Sch., and *Alectorobius tholozan papillipas* Birula). Evaluated were butylacetanilide, tetrahydroquinoline, a mixture of ethyleneoxide–carbon dioxide (1:9)[1], dibutyl adipate, dimethyl phthalate, *N,N*-diethyl-*m*-toluamide benzimide, isoamyl acetamide, and benzoyl piperidine.

In the present study, a combination of the amide function with the ethylene glycol moiety were examined for repellent activity against ticks.

EXPERIMENTAL[2]

2-(Hydroxyethyoxy)acetamides—2-(2-Hydroxyethoxy) - *N*, *N* - diisopropylacetamide was prepared as follows: Sodium (1.4 g, 0.006 mole) was dissolved in 13 ml (0.24 mole) of ethylene glycol. After cooling to room temperature, 12 g (0.0676 mole) of *N,N*-diisopropyl-chloroacetamide (prepared from chloroacetylchloride and diisopropylamine) was added. The mixture was stirred at 90° for 1 hr. The ethylene glycol was distilled under reduced pressure and the residue taken up in ether, filtered to remove the sodium chloride, evaporated *in vacuo*, and distilled[3] to give 10.6 g of product, 125° air bath temperature/0.8 mm Hg.

2,2′-Ethylenedioxy-bis(*N,N* - dialkylacetamides)—2,2′- Ethylenedioxy-bis(*N,N*-diisopropylacetamide) was prepared as follows: A 150-ml three-necked flask was fitted with a stirrer, a reflux condenser, and a dropping funnel. Sodium (0.78 g, 0.034 mole) was suspended by vigorous stirring in 20 ml of boiling xylene. Ethylene glycol (1.05 g, 0.017 mole) was dropped slowly into the sodium suspension at reflux temperature, the suspension stirred and refluxed for an additional 7 hr, and then 6 g (0.034 mole) of *N,N*-diisopropyl-chloroacetamide in 15 ml of xylene was dropped into the stirred suspension at reflux temperature during 1 hr. The reaction mixture was refluxed and stirred for an additional hour.

[1] Carboxide.

[2] Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

[3] Distilled with a Kugelrohr.

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