*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 4, No. 4, pp. 517–521, 1986
Printed in Great Britain

0731–7085/86 $3.00 + 0.00


*Short Communication*

# Use of circular dichroism spectroscopy for the determination of oily injections containing a $\Delta^4$-3-ketosteroid*

A. GERGELY† and GY. SZÁSZ

*Department of Pharmaceutical Chemistry, Semmelweis Medical University, H-1092 Budapest, Högyes Endre u. 9, Hungary*

**Keywords**: *CD spectroscopy; 4-en-3-one-chromophore; oily injection; steroid hormones; testesterone phenylpropionate; nandrolone phenylpropionate; progesterone.*

## Introduction

The low-intensity long-wavelength absorption band of α, β-unsaturated ketones varies considerably with change in the polarity of the solvent [1, 2]. The fine structure of the absorption band arising reflecting from the $n \rightarrow \pi$ electron transition in $\Delta^4$-3-ketosteroids can be better resolved and measured with greater sensitivity by means of chiroptical methods than with electron excitation spectrophotometry. The selectivity of analytical methods for these steroids is improved if use is made of the solvent dependence of the circular dichroism (CD) exhibited by the compounds. A difference CD spectroscopic procedure based upon this principle has been described [3].

Steroid hormones containing the $\Delta^4$-3-keto group frequently are marketed in the form of oily injections. The present CD method involves the direct quantitative determination of the active ingredient by measurement of the ellipticity of the injection after dilution with dioxan.

## Experimental

The steroid reference samples and oily injections were provided by the Richter Gedeon Pharmaceutical Works, Hungary. The specific rotation values of the steroids were checked with a Perkin–Elmer 241 MC polarimeter.

Dioxan, cyclohexane (Reanal, Budapest) were purified by distillation after treatment with potassium hydroxide and then with sodium.

* This is part X of the series "Application of chiroptical methods in drug analysis". For part IX of the series, see *Fresenius Z. Anal. Chem.* **318**, 528–530 (1984).

† To whom correspondence should be addressed.

A Jasco 40C dichrograph was used to measure ellipticity; it was calibrated with D-camphor-10-sulphonic acid (EGA Chemie) at 290 nm [4].

*Procedure*

A 0.5 ml injection was diluted to 25 ml with dioxan in a volumetric flask. The solution was thoroughly mixed and then transferred to a 1-cm Jasco quartz cell and thermostated at 25 ± 0.1 °C. The ellipticity of the solution was measured at 342.8 nm against dioxan. The ellipticity of a standard injection containing a known concentration of the steroid was also measured and the concentration of the steroid in the sample injection was calculated from the proportional relationship that exists between ellipticity and concentration.

## Results and Discussion

In an earlier study [3], the CD and difference CD spectra of $\Delta^4$-3-ketosteroids in different solvents were reported. Of the solvents studied, cyclohexane proved to be the most suitable. However, in this study, it was found that benzyl alcohol, present in the oily injection as a preservative, at a concentration of 10% v/v, distorts the CD spectrum of testosterone phenylpropionate when cyclohexane is used as the solvent. The variation of the CD spectra of testosterone phenylpropionate in the presence of increasing concentrations of benzyl alcohol is shown in Fig. 1. The degree of distortion in the spectra is demonstrated by plotting the ratio of ellipticities at the wavelengths of maximum negative ellipticity around 320 and 350 nm against the concentration of benzyl alcohol in the injection (Fig. 2a).

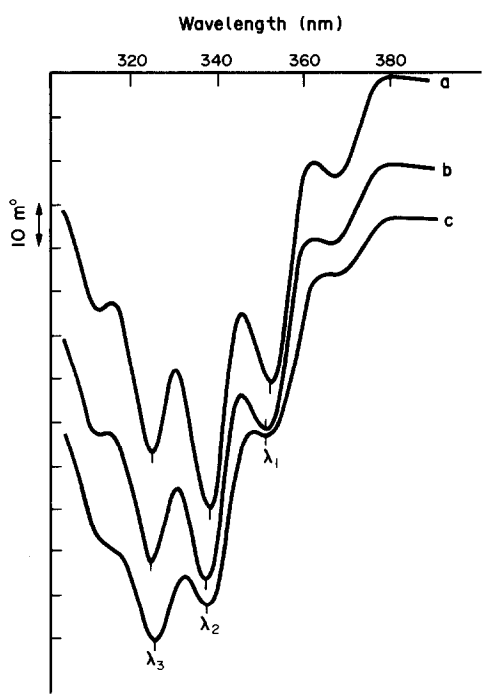


**Figure 1**
CD curves of testosterone phenylpropionate injections with various concentrations of benzyl alcohol. A 1.0 ml injection was diluted to 25.0 ml with cyclohexane. (a) 50 μl, (b) 200 μl, (c) 600 μl benzyl alcohol per 1 ml oily solution. The descending ordinate represents increasing negative ellipticity and the scale divisions are 0.01°.

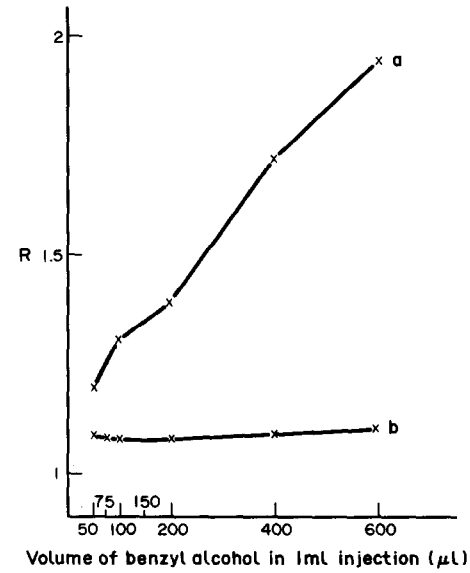


**Figure 2**
Dependence of the ellipticity ratio ($R$) of testosterone phenylpropionate on the benzyl alcohol content of injection after dilution with (a) cyclohexane and (b) dioxan.

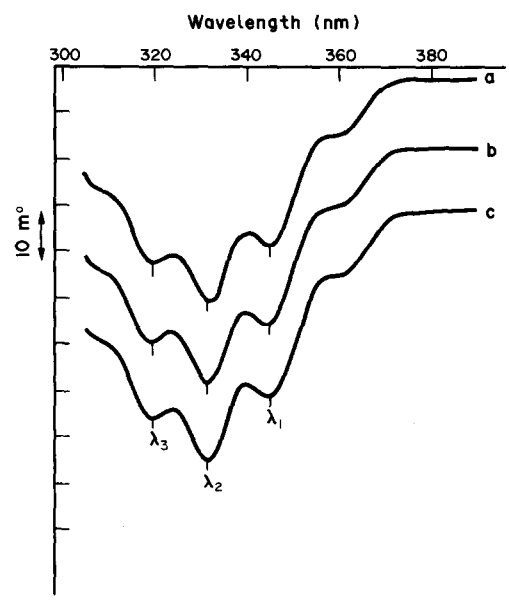


**Figure 3**
CD curves of testosterone phenylpropionate injections with various concentrations of benzyl alcohol. A 0.5 ml injection diluted to 25.0 ml with dioxan. (a) 50 μl, (b) 200 μl, (c) 600 μl benzyl alcohol per 1 ml oily solution. The descending ordinate represents increasing negative ellipticity and the scale divisions are 0.01°.

The dependence of the CD spectra of testosterone phenylpropionate in other solvents upon the concentration of benzyl alcohol was investigated. Dioxan was found to be the most suitable solvent as no significant distortion of the CD spectra occurred in the presence of increasing concentrations of benzyl alcohol (Figs 2b and 3).

Sunflower oil, the solvent for the steroids in the oily injection formulations, was found to give negative ellipticity below 334 nm which interferes with that of testosterone phenylpropionate at its wavelength of maximum negative ellipticity, 330 nm. Consequently, the wavelength selected for the direct measurement of ellipticity of the

steroids was 342.8 nm, the long wavelength of maximum negative ellipticity of the steroids, where the sunflower oil exhibits no circular dichroism.

The ellipticity of an oily injection of testosterone phenylpropionate, diluted with dioxan, was found to be proportional to the concentration of the steroid in the range 0.04–0.7 mg $ml^{-1}$: $y = -73.5x - 0.07$, where $y$ is the measured ellipticity at 342.8 nm in millidegrees and $x$ is the concentration in mg $ml^{-1}$ ($n = 22$; $r = -0.9994$). Similar proportional relationships were found to exist for oily injections of nandrolone phenylpropionate ($y = 76.97x - 0.06$; $n = 18$, $r = -0.9995$) and progesterone ($y = -100.5x - 0.06$; $n = 18$, $r = -0.9994$).

The procedure was applied to Retandrol® Injection containing testosterone phenylpropionate, Nerobolil® Injection containing nandrolone phenylpropionate, Glanducorpin® Injection containing progesterone, and Limovanil® Injection containing progesterone and oestradiol benzoate. The oestrogen component in Limovanil Injection was shown to have no effect on the ellipticity of progesterone. For comparison, the concentration of the 4-en-3-one steroids in the oily injections was also determined by the difference spectrophotometric method of Görög [5]. The results in Table 1 for standard injections which contain known amounts of the steroids, and for commercial samples, show that good agreement was obtained between the concentrations determined by

**Table 1**
Results for model* and commercial injections assayed by the CD spectroscopic method and difference spectrophotometric (ΔA) method

| Concentration of steroid (mg $ml^{-1}$) | | | |
|---|---|---|---|
| | | Found | |
| | Taken | CD method† | ΔA method [5] |
| Testosterone phenylpropionate in Retandrol | 8.97 | 8.89 ± 0.11‡ | 8.77 |
| | 15.02 | 15.15 ± 0.22‡ | 15.11 |
| | 25.02 | 25.04 ± 0.31‡ | 25.12 |
| | 25§ | 25.58 ± 0.27‡ | 25.47 |
| | 25§ | 25.05 ± 0.25 | 24.83 |
| Nandrolone phenylpropionate in Nerobolil | 6.38 | 6.32 ± 0.10‡ | 6.24 |
| | 17.68 | 17.62 ± 0.18‡ | 17.71 |
| | 23.22 | 23.33 ± 0.22‡ | 23.07 |
| | 25§ | 25.10 ± 0.26 | 25.28 |
| | 25§ | 25.36 ± 0.26‡ | 25.07 |
| Progesterone in Glanducorpin | 7.32 | 7.25 ± 0.16‡ | 7.41 |
| | 16.29 | 16.28 ± 0.10‡ | 16.37 |
| | 25.87 | 25.96 ± 0.12‡ | 25.53 |
| | 10§ | 10.10 ± 0.06‡ | 10.18 |
| | 25§ | 25.33 ± 0.13‡ | 25.54 |
| | 25§ | 24.83 ± 0.15 | 24.87 |
| Progesterone in Limovanil | 9.78 | 9.78 ± 0.12‡ | 9.69 |
| | 15.88 | 15.65 ± 0.13‡ | 15.71 |
| | 12.5§ | 12.83 ± 0.10‡ | 12.67 |
| | 12.5§ | 12.63 ± 0.11‡ | 12.53 |

*The active ingredient content of model injections was varied.
†Calculated from six determinations.
‡Confidence interval at the 95% probability level.
§Declared values of commercial injections.

measurement of ellipticity and the quantity of steroid added to the standard injections or declared in the commercial samples. The good agreement of the results with those obtained by using the difference spectrophotometric procedure further confirms the accuracy of the direct CD assay of $\Delta^4$-3-ketosteroids.

## References

[1] A. E. Lippmann, E. W. Foltz and C. D. Djerassi, *J. Am. Chem. Soc.* **77**, 4364–4367 (1955).
[2] C. Djerassi, R. Riniker and E. Riniker, *J. Am. Chem. Soc.* **78**, 6377–6389 (1956).
[3] A. Gergely and Gy. Szász, *Acta Pharm. Hung.* **53**, 280–287 (1983).
[4] K. Tuzimura, T. Konno and M. Meguro, *Anal. Biochem.* **31**, 167–174 (1977).
[5] S. Görög, *J. Pharm. Sci.* **57**, 1737–1741 (1968).

[*First received for review 26 October 1984; resubmitted 3 January 1985*]