The specificity of the method was determined with dosage form samples maintained for 6 months at room temperature, 37°, and 45°. The results are given in Table II.

RESULTS AND DISCUSSION

A microbiological method for the determination of neomycin and spiramycin mixed in pharmaceutical preparations was developed. The initial plan was to develop a *Staphylococcus* strain that was resistant to spiramycin for the determination of neomycin and to develop a *Bacillus* strain that was resistant to neomycin for the determination of spiramycin.

Resistance to spiramycin in *S. aureus* was obtained easily. However, since the *B. subtilis* inoculum was a spore suspension, a strain resistant to neomycin was not obtained. Therefore, since *S. aureus* also is recommended for the evaluation of spiramycin (4), this microorganism was chosen for both antibiotics.

This method may be used as a quality control procedure for samples of production batches as well as in stability studies since substances produced by degradation products of excipients or other active ingredients do not interfere. The assay may be carried out without special preparation of the sample. This technique may be used in the assay of mixtures of other antibiotics by inducing resistance in the proper microorganisms.

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Determination of Dextro- and Levomethorphan Mixtures Using Chiral Lanthanide NMR Shift Reagents

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Abstract □ Europium and praseodymium chiral NMR shift reagents were used to differentiate between dextro- and levomethorphan. The enantiomeric shift differences ($\Delta\Delta\delta$) demonstrated by the singlet associated with the methoxy protons were large enough to identify the *levo*- and *dextro*-isomers and to allow for the determination of as little as 10% of one enantiomer in the presence of the other in a prepared mixture. The analytical method is rapid and can analyze samples as small as 1 mg.

Keyphrases □ Dextromethorphan—NMR determination with levomethorphan □ Levomethorphan—NMR determination with dextromethorphan □ Enantiomers—NMR determination of dextromethorphan and levomethorphan mixtures, chiral lanthanide shift reagents □ NMR spectroscopy—determination of dextro- and levomethorphan mixtures, chiral lanthanide shift reagents □ Analgesics, narcotic—levomethorphan, NMR identification with dextromethorphan □ Antitussives, nonnarcotic—dextromethorphan, NMR identification with levomethorphan

The determination of the enantiomeric purity of a substance is an important aspect of the regulatory process. This determination is especially important in cases such as methorphan where the *levo*- and racemic forms of the drug are regulated narcotics while the *dextro*-isomer is a widely used nonnarcotic antitussive.

BACKGROUND

Specific rotation (1) is the standard method used to distinguish dextromethorphan from levomethorphan. This method is simple and accurate if there is an adequate amount of sample and if one can be reasonably certain that there are no interfering compounds. The hanging microdrop technique (2), which has a sensitivity of 0.2 μg, also is used. However, these approaches cannot serve as identification tests and they cannot accurately detect one enantiomer in the presence of the other.

Chiral lanthanide nuclear magnetic shift reagents may be utilized in the development of rapid, sensitive, and accurate assays of enantiomeric purity. Shaath and Soine (3) recently demonstrated the utility of this approach in the determination of the enantiomeric purity of isoquinoline alkaloids. By using a europium chiral shift reagent, they differentiated rapidly between the components of five enantiomeric pairs and accurately detected an enantiomeric mixture of 95:5.

This paper reports the application of the chiral lanthanide shift reagents to the identification of the methorphan enantiomers and the determination of one isomer in the presence of the other.

EXPERIMENTAL

Instrumentation—A 60-MHz NMR spectrometer[1] equipped with a Fourier transform system[2] was used for all spectra.

Reagents and Chemicals—Deuterated chloroform was used as purchased[3].

Dextro- and levomethorphan were supplied as their hydrobromide salts[4], and the free bases were prepared according to the NF procedure (1). Stock solutions (0.16 *M*) of the free bases were prepared by dissolving 220 mg of the respective enantiomer in 5.00 ml of deuterated chloroform. Enantiomeric mixtures were prepared from the two stock solutions.

The europium and praseodymium chiral shift reagents were used as purchased[5]. Stock solutions (0.2 *M* in deuterated chloroform) were prepared in a dry box under a nitrogen atmosphere before the start of each series of experiments. Each solution was stored in a sealed vial equipped with a septum and sampled using a microliter syringe.

Procedure—The appropriate methorphan solution (0.4 ml) was placed in an NMR tube equipped with a rubber septum. The shift reagent then was added sequentially in 20-μl increments. An NMR spectrum with 64 additive pulses was run after each addition, and the peak chemical shifts were computer recorded.

The peak height ratios associated with the various molar ratios of levo- and dextromethorphan were measured after the addition of 300 μl of the europium shift reagent or 260 μl of the praseodymium shift reagent.

RESULTS AND DISCUSSION

The effect of the europium shift reagent on the NMR spectrum of an equimolar mixture of dextro- and levomethorphan is shown in Fig. 1. The

[1] Model R12B, Perkin-Elmer Corp., Norwalk, CT 06856.
[2] TT7, Nicolet Instrument Corp., Madison, WI 53711.
[3] Aldrich Chemical Co., Milwaukee, WI 53233.
[4] Hoffmann-La Roche, Nutley, NJ 07110.
[5] Eu-Opt and Pra-Opt, Alfa Products, Ventron Corp., Danvers, MA 01923.

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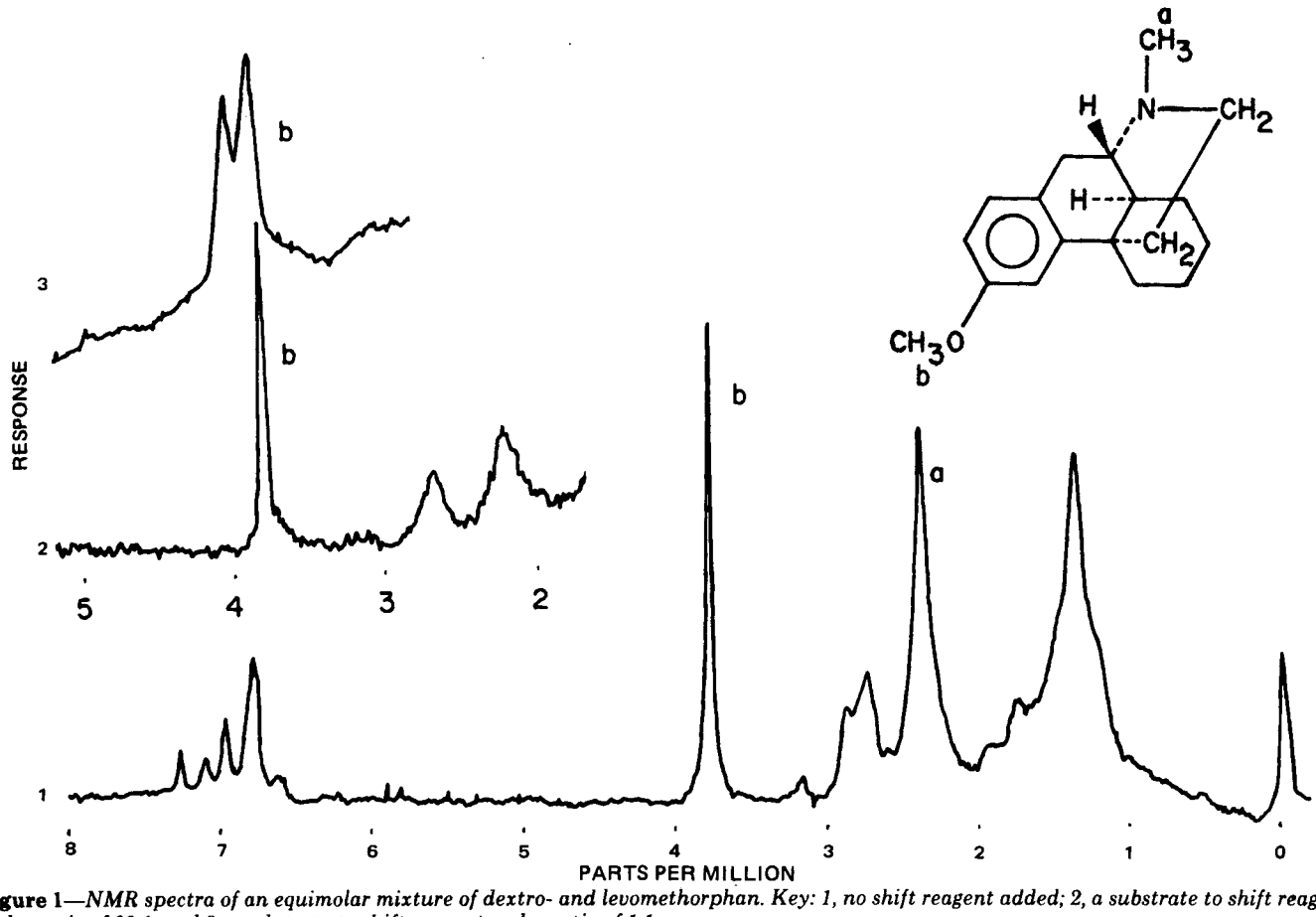

Figure 1—*NMR spectra of an equimolar mixture of dextro- and levomethorphan. Key: 1, no shift reagent added; 2, a substrate to shift reagent molar ratio of 80:1; and 3, a substrate to shift reagent molar ratio of 1:1.*

singlet associated with the N-methyl protons at 2.9 ppm broadened and disappeared at a substrate to reagent molar ratio of 80:1. This result suggests that the nitrogen atom is the site of pseudocontact or dipolar interaction between the methorphan and the shift reagent.

The induced shifts ($\Delta\delta$) and the enantiomeric shift differences ($\Delta\Delta\delta$) for the singlet due to the methoxy protons are shown in Figs. 2 and 3, respectively. The europium shift reagent had little effect on the spectra up to a substrate to shift reagent molar ratio of 8:3. Continued addition of the shift reagent shifted the signal associated with the *dextro*-isomer downfield while the signal associated with the *levo*-isomer remained relatively constant. The maximum resolution of the signals occurred at a substrate to reagent molar ratio of ~1:1. Beyond this point, peak broadening tended to collapse the peaks. The signal from the *dextro*-isomer was the one most affected by this broadening, with the result that the peak height associated with dextromethorphan was 85% of the corresponding peak height for levomethorphan at a 50:50 ratio of the two isomers.

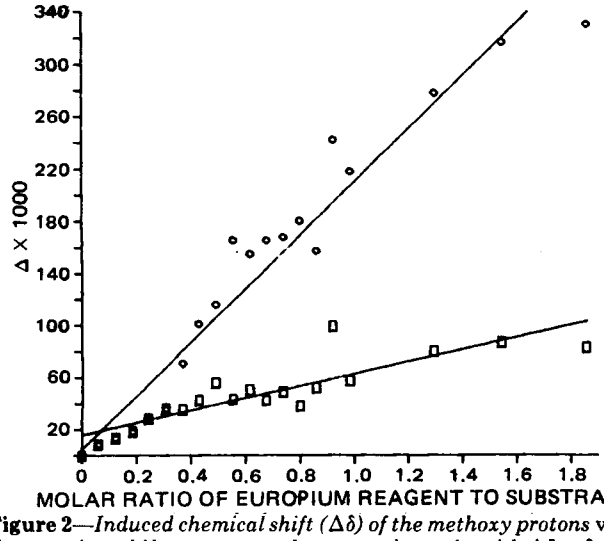

Figure 2—*Induced chemical shift ($\Delta\delta$) of the methoxy protons* versus *the europium shift reagent to substrate molar ratio, with $\Delta\delta = \delta t - \delta_0$, where δ_0 is the chemical shift of the initial signal and δt is the chemical shift of the signal after the addition of the shift reagent. Key:* ○, *dextromethorphan; and* □, *levomethorphan.*

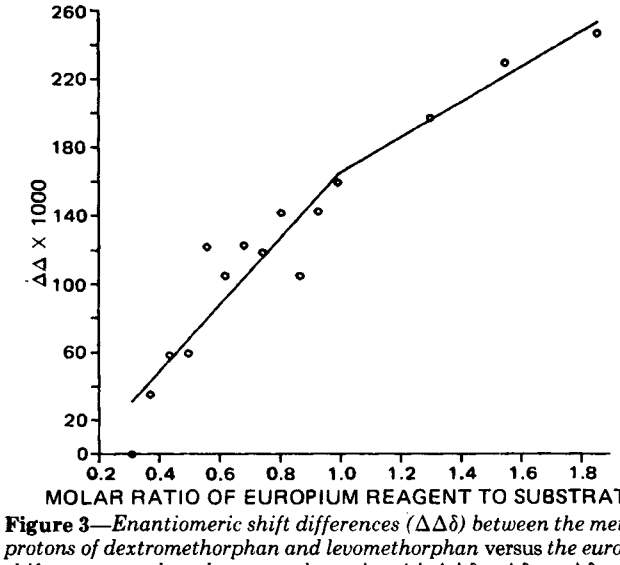

Figure 3—*Enantiomeric shift differences ($\Delta\Delta\delta$) between the methoxy protons of dextromethorphan and levomethorphan* versus *the europium shift reagent to the substrate molar ratio, with $\Delta\Delta\delta = \Delta\delta_D - \Delta\delta_L$, where $\Delta\delta_D$ is the induced chemical shift for the methoxy protons of dextromethorphan and $\Delta\delta_L$ is the induced chemical shift for the methoxy protons of levomethorphan.*

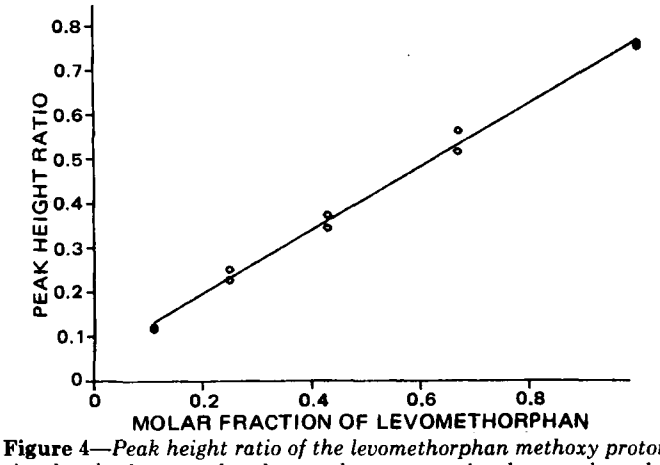

Figure 4—*Peak height ratio of the levomethorphan methoxy proton signal to the dextromethorphan methoxy proton signal* versus *the molar fraction of levomethorphan in dextromethorphan.*

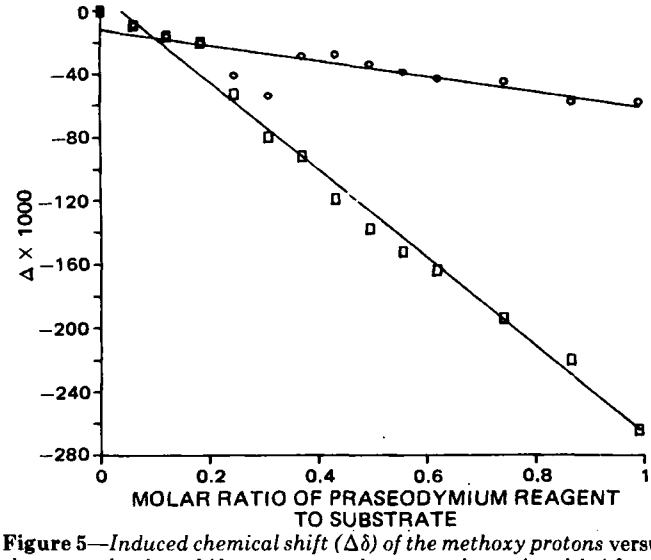

Figure 5—*Induced chemical shift ($\Delta\delta$) of the methoxy protons* versus *the praseodymium shift reagent to substrate molar ratio, with $\Delta\delta = \delta t - \delta_0$, where δ_0 is the chemical shift of the initial signal and δt is the chemical shift of the signal after the addition of the shift reagent. Key:* O, *levomethorphan; and* □, *dextromethorphan.*

The peak height ratios that correspond to prepared solutions of various molar ratios of the two enantiomers are shown in Fig. 4. The peak heights were determined by direct measurement to an adjusted baseline. The peak heights of shoulders were determined by dropping a vertical line to intersect the tangent of the slope of the larger peak. In this manner, as little as 10% of the *levo*-isomer could be estimated in the presence of the *dextro*-isomer. At this level, the signal associated with the *levo*-isomer appeared as a shoulder of the signal associated with the *dextro*-isomer, with a peak height of ~1:10.

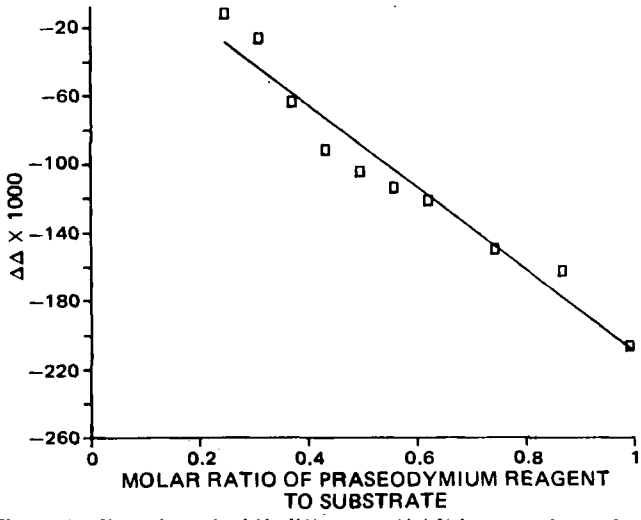

Figure 6—*Enantiomeric shift differences ($\Delta\Delta\delta$) between the methoxy protons of dextromethorphan and levomethorphan* versus *the praseodymium shift reagent to substrate molar ratio, with $\Delta\Delta\delta = \Delta\delta_D - \Delta\delta_L$, where $\Delta\delta_D$ is the induced chemical shift for the methoxy protons of dextromethorphan and $\Delta\delta_L$ is the induced chemical shift for the methoxy protons of levomethorphan.*

The induced shifts ($\Delta\delta$) and the enantiomeric shift differences ($\Delta\Delta\delta$) of the methoxy protons after the addition of the praseodymium reagent are shown in Figs. 5 and 6, respectively. The effects of this reagent on the spectra of the *dextro*- and *levo*-isomers were similar to those observed with the europium reagent, except that the signal associated with dextromethorphan was shifted upfield. The maximum resolution between the two methoxy signals occurred at a substrate to reagent molar ratio of 10:8. The peak height ratios associated with the various molar ratios of the two enantiomers also were similar to those determined with the europium shift reagent. However, the peak broadening associated with the praseodymium reagent was greater than that observed with the europium reagent, making the praseodymium reagent less sensitive as an analytical probe for the detection of one isomer in the presence of the other.

In conclusion, the chiral lanthanide shift reagents can be used to identify the enantiomers of methorphan. This method rapidly and accurately differentiates between dextromethorphan and levomethorphan, with detection and estimation of as little as 10% of one isomer in the presence of the other.

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