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# LIQUID CHROMATOGRAPHIC ANALYSIS OF ENANTIOMERIC PURITY OF SEVERAL TERPENOID ACIDS AS THEIR 1-(1-NAPHTHYL)ETHYLAMIDE DERIVATIVES

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## SUMMARY

A rapid high-resolution liquid chromatographic (HRLC) analysis for enantiomeric purity of chiral monoterpenoid acids is described. The acids are converted to diastereomeric amides using commercially available (+)-1-(1-naphthyl)ethylamine. The amides are chromatographed on microparticulate silica HRLC columns with ultraviolet detection at 254 nm; the enantiomeric composition is then derived from the diasteromeric ratios. Good separation of various pairs of diastereomers are reported, with very low loadings ($\leqslant 1$ $\mu$g) required because of high molecular extinction coefficients. No conclusive correlations between elution order and absolute configuration could be determined.

## INTRODUCTION

Optical purity is classically measured by comparing the rotation of a sample to the rotation of "optically pure" reference material. This technique is accurate only for samples of relatively low optical purity. Enantiomeric purity, and hence optical purity, can be determined by a direct measurement of the proportions of the enantiomers present in a mixture. Determination of the presence of a few percent of one antipode contaminating another has become possible by NMR methods using diastereomeric derivatives[1] or optically active shift reagents[2]. However, for quantitative purposes chromatographic methods offer greater sensitivity of detection of minor components than do NMR methods. While reports of gas chromatographic resolution of racemates on columns prepared with chiral stationary phases do exist[3], a more popular method involves preparation of pairs of diastereomeric derivatives, whose differing physical properties enable separations[4,5] on common gas–liquid chromatographic (GLC) phases.

High-resolution liquid chromatography (HRLC) has potential advantages for separation of diastereomeric derivatives, since many such derivatives are of relatively high molecular weight and may be thermally unstable on GLC analysis. Also, both analysis and quantitative semipreparative isolation are feasible by HRLC. We have long been interested in this technique, using it for analysis of the optical purity of

the synthetic unnatural antipode of the insect juvenile hormone JH I (*Cecropia* $C_{18}$JH)[6] and for a micro-method of determining the absolute configuration of the third known juvenile hormone JH III (ref. 7).

Recently we needed to determine the enantiomeric purity of certain terpenoid aldehydes used in the synthesis of insect growth regulators[8], since biological assays on various insect species revealed large differences in activity between the (+)- and (−)-antipodes of the latter compounds[8,9].

Attempts to determine optical purity of citronellol or citronellic acid by analysis of diastereomeric ester mixtures were unsuccessful. Thus, the esters from (±)-citronellol and (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride could not be resolved by GLC or HRLC, and the proton and fluorine NMR spectra showed no resolution of the diastereomer signals. Addition of shift reagents to the NMR sample also did not give satisfactory resolution of the signals. Likewise, the esters of (±)-citronelloyl chloride and L-menthol were not separable by GLC analysis.

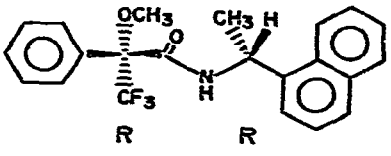
Valentine *et al.*[10] and Scott *et al.*[11] have reported the resolution of certain terpenoid acids as their α-methyl-*p*-nitrobenzylamides. Since the optically active amine used for their resolutions is not commercially available, we sought to develop a method using reagents that were readily available in high optical purity. We find that 1-phenylethylamine, and especially 1-(1-naphthyl)ethylamine, are useful reagents for conversion of terpenoid acids to derivatives that are separable by HRLC. Herein we report the details of derivatization and separation.

## EXPERIMENTAL

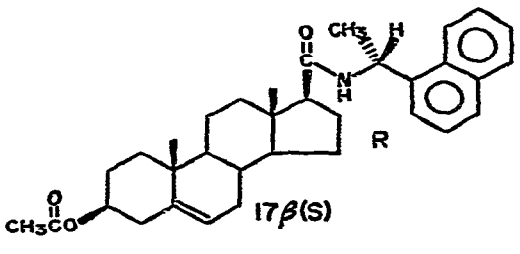
### *Purification and analysis of chiral amine*

An enantiomerically pure sample of (+)-1-(1-naphthyl)ethylamine was prepared from commercial material (*ca.* 93% optical purity; Cyclo Chemicals, Los Angeles, Calif., U.S.A.) by recrystallization of the bitartrate salt. A 0.13 *M* solution of equimolar amounts of (+)-amine and L-tartaric acid (J. T. Baker, Phillipsburgh, N.J., U.S.A.) in 94% methanol was heated to 55–60°. Overnight cooling to room temperature gave crystals which were filtered and subsequently reconverted to free amine by extraction into diethyl ether from aqueous base.

The (+)-form of the amine resolving agent is reported to have the *R*-configuration, based upon X-ray crystallography studies[12]. In order to check the enantiomeric purity of the amine, it was converted to the amide I or II, using the acid chlorides of (+)-α-methoxy-α-trifluoromethylphenylacetic acid (Aldrich, Milwaukee, Wisc., U.S.A.) and 3β-acetoxyetienic acid (Steraloids, Wilton, N.H., U.S.A.) respectively.




I

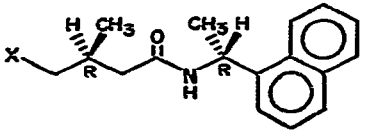



II

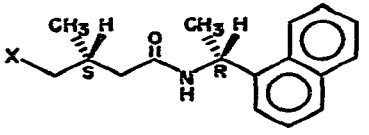
Following thin-layer chromatographic (TLC) purification, the amides were then analyzed by HRLC to obtain the purity values cited.

*Formation of derivatives*

Typically, (3*RS*)-citronellol was oxidized to the corresponding acid by an excess of Jones reagent. The recovered acid was then treated with thionyl chloride in diethyl ether containing a catalytic amount of dimethylformamide to generate the acid chloride. Treatment with excess enantiomerically pure (*R*)-(+)-1-(1-naphthyl)-ethylamine gave the crude diastereomeric amides III*, which were purified by preparative TLC before HRLC analysis. (3*RS*)-7-Methoxycitronellic acid was synthesized via methoxymercuration of (±)-citronellol, and converted to the amide V as above. (3*RS*)-Dihydrocitronellic acid was prepared via hydrogenation of (±)-citronellol, and treated as above to ultimately produce the amide IV.

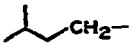


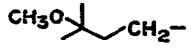
($R_{ac}$, R)



($S_{ac}$, R)

III X= 

IV X= 

V X= 

*Analytical procedure*

The liquid chromatograph consisted of a Haskel Model 26920-4 pneumatic amplifier pump, a Valco Model CV-6-HPax loop injector and a Chromatronix Model 230 UV absorbance detector set on the 254-nm channel. All separations were performed on a single Zorbax-SIL (DuPont, Wilmington, Del., U.S.A.) silica column (22 × 0.46 cm I.D.) using 14 or 22% ethyl acetate in pentane. Solvents were either

* $R_{ac}$ (or $S_{ac}$) designates the absolute configuration of the acyl moiety of the diastereomeric amides, while the *R* refers to the amino moiety.

TABLE I

CHROMATOGRAPHIC PARAMETERS OF VARIOUS 1-(1-NAPHTHYL)ETHYLAMIDES

| *Parameter* | *Compound I* | | *Compound II* | | *Compound III* | | *Compound IV* | | *Compound V* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *($R_{ac}$,S)* | *($R_{ac}$,R)* | *[17β(S)$_{ac}$,R]* | *[17β(S)$_{ac}$,S]* | *($R_{ac}$,R)* | *($S_{ac}$,R)* | *($R_{ac}$,R)* | *($S_{ac}$,R)* | *($S_{ac}$,R)* | *($R_{ac}$,R)* |
| *N* | 13000 | 13800 | 7900 | 8600 | 7300 | 8100 | 7500 | 9200 | 7600 | 8900 |
| *k'* | 5.8 | 7.1 | 3.2 | 7.2 | 6.6 | 8.0 | 5.5 | 6.8 | 16.5 | 18.0 |
| $R_s$ | 5.0 | | 13 | | 3.8 | | 4.2 | | 1.9 | |
| α | 1.22 | | 2.25 | | 1.21 | | 1.24 | | 1.09 | |
| *Eluent* | pentane–ethyl acetate (95:5), "100% water-saturated" | | pentane–ethyl acetate (80:20), "100% water-saturated" | | pentane–ethyl acetate (86:14), "50% water-saturated" | | pentane–ethyl acetate (86:14), "50% water saturated" | | pentane–ethyl acetate (78:22), "100% water-saturated" | |

50% or 100% saturated with water. Diastereomeric ratios were determined by peak area computation.

Table I shows important chromatographic parameters for the separation of the diastereomeric pairs studied. The commercially available column exhibited excellent $N$ values (equivalent to $\geqslant$35,000 plates per meter). The resolution factor $R_s$ greatly exceeds unity in all cases.

## RESULTS AND DISCUSSION

Our initial efforts were directed toward the resolution of diastereomeric $\alpha$-phenylethylamides of dihydrocitronellic acid. However, because of the low UV extinction coefficient of these derivatives at 254 nm, very high column loadings were required, which decreased the resolution markedly. We therefore investigated the utility of 1-(1-naphthyl)ethylamides as diastereomeric derivatives, because of the much higher $\varepsilon_{254}$ value of the naphthalene ring. In addition to a significant increase in sensitivity of detection, we also obtained a gain in selectivity of separation, resulting in increased resolution of the diastereomeric pairs.

If a highly sensitive limit of detection of the minor terpenoid acid antipode is desired, careful purification and subsequent analysis of the $R$-1-(1-naphthyl)ethylamine is mandatory. The commercial amine contained varying amounts of the $S$-antipode (3.6% in a typical batch), and must be further purified as the bitartrate salt. A single crystallization of this salt brought the enantiomeric purity up to $\geqslant$99.9%, as determined by HRLC analysis of I or II. For these purity checks we prepared the corresponding derivatives from the $S$-amine for determination of the $k'$ value of the minor diastereomers.

Since the conditions used for amide formation generate impurities, a preliminary TLC clean-up was required. While selective removal of one diastereomer is the usual aim of a resolution procedure, it is highly undesirable for a procedure designed for enantiomeric purity analysis. Therefore, particular care was taken to avoid selective removal of one diastereomer from the other during this step. As the derivatives are crystalline, we were also careful to prepare dilutions for analysis from a solution of the entire sample, since a single crystal might conceivably be greatly enriched in one diastereomer. Also, excess amine was used to prevent kinetic resolution effects which might be expected in a reaction with antipodal acyl chlorides.

### *Enantiomeric purity analysis of unsubstituted isoprenoid acids*

Very rapid and complete resolution of the ($R_{ac}$, $R$) and ($S_{ac}$, $R$) 1-(1-naphthyl)-ethylamides was achieved in the citronellic and dihydrocitronellic acid series. Fig. 1 shows the analysis of III in which there is baseline resolution in a total time of analysis of 14 min. Similar resolution (in 11 min) is obtained for IV (Fig. 2). Clearly, diastereomeric separations in the above two series can be achieved in well under 10 min each (at higher flow-rates) without unduly affecting resolution.

As an illustration of the method, ($R$)-(+)-pulegone (Givaudan, Clifton, N. J., U.S.A.; 96% chemical purity), $[\alpha]^{25} + 24.57°$ (neat), was converted to ($R$)-(+)-citronellic acid, $[\alpha]^{25} + 8.44°$ (neat) according to the procedure of Overberger and Weise[13]. HRLC analysis of the diastereomeric amides derived from this sample of (+)-citronellic acid and ($R$)-(+)-1-(1-naphthyl)ethylamine demonstrated the acid to be of 99.5%

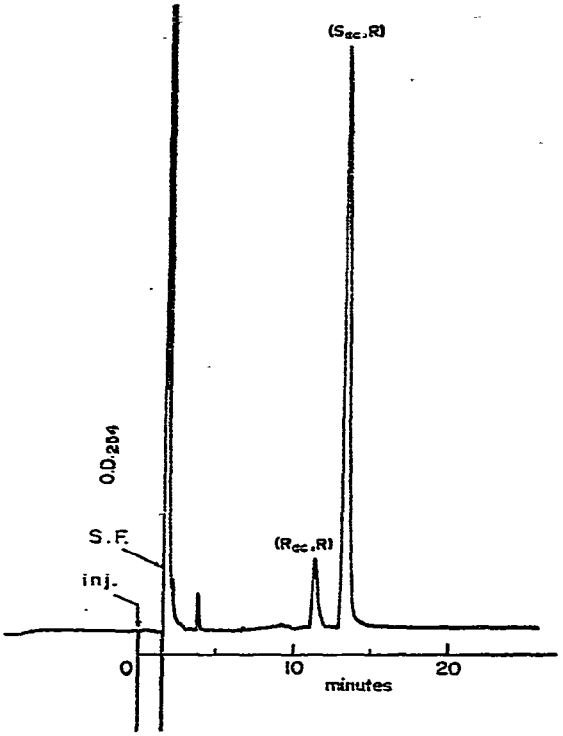



Fig. 1. HRLC analysis of diastereomeric amides III from citronellic acid and (*R*)-(+)-1-(1-naphthyl)-ethylamine. Conditions: Zorbax-SIL (22 × 0.46 cm I.D.) column, eluted with pentane–ethyl acetate (86:14) ("50% water-saturated"), 1.8 ml/min at 400 p.s.i.g.

enantiomeric purity (*i.e.*, enantiomeric composition of 99.75% *R* and 0.25% *S*). Reduction of this sample of (*R*)-(+)-citronellic acid with $LiAlH_4$ gave (*R*)-(+)-citronellol (99% chemical purity), $[\alpha]^{23} + 5.49°$ (neat).

### *7-Methoxycitronellic acid analysis*

In the alkoxyl substituted series, separation of the now more polar diastereomers is more difficult and speed of analysis is sacrificed for resolution. Fig. 3 illustrates the decreased separation of diastereoisomers of V compared to unsubstituted isoprenoid acid derivatives. A key point is a reversal of elution order between the diastereomers; in the alkyl and alkenyl series the ($R_{ac}$, *R*) diastereomer eluted first, while in the alkoxyl series the ($S_{ac}$, *R*) diastereomer was the faster eluting. Scott *et al.*[11] had observed no inversion of HRLC elution patterns from homologous series of isoprenoid acid (*R*)-α-ethyl-*p*-nitrobenzylamide pairs, including ω-*tert.*-butoxyl analogs of the carboxylic acids. In the latter case, the authors noted a decrease of α in *tert.*-butoxyl *vs.* hydrogen substitution, though not as pronounced as we observed for methoxyl *vs.* hydrogen.

An equally good separation in the alkoxyl series is obtained by using a 0.13% acetonitrile modifier in place of water saturation, and concomitantly reducing ethyl acetate content by *ca.* 10%. The addition of acetonitrile reduces tailing somewhat,

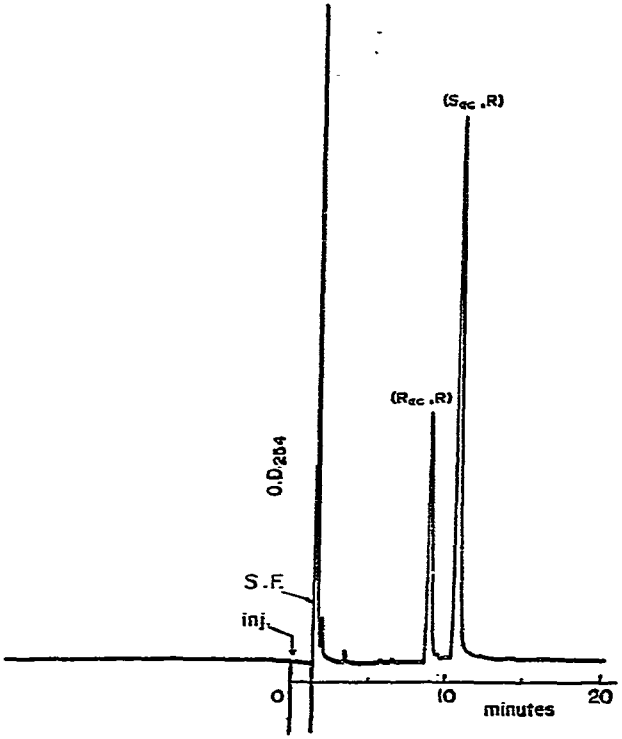



Fig. 2. HRLC analysis of diastereomeric amides IV from dihydrocitronellic acid and (*R*)-(+)-1-(1-naphthyl)ethylamine. Eluent flow-rate, 2.2 ml/min at 480 p.s.i.g. Other conditions as in Fig. 1.

but the modifier content is critical, as serious loss of column selectivity results at higher acetonitrile concentrations. Diastereomeric separation was *appreciably* diminished when a diethyl ether–pentane system was tried. Further manipulation of solvents may be desirable, as individual differences in selectivity of the above separation were observed among three Zorbax-SIL columns of comparable efficiency and identical manufacturer's specifications. The data reported herein were obtained from the most selective of the columns*.

*Prediction of elution order*

The apparently "anomalous" elution order of diastereomers of V illustrates the inherent difficulties encountered if one wishes to formulate a predictive theory of elution patterns based on absolute configuration of diastereomeric pairs chromatographed on achiral liquid chromatographic adsorbents. A certain degree of success has been achieved in predicting elution order of enantiomeric pairs chromatographed on achiral LC supports by Pirkle and Sikkenga[14], based upon substrate–support stereochemically mediated interactions. Also, Helmchen *et al.*[15] have recently claimed

* Excellent resolution ($\alpha = 1.09$, $R_s = 1.5$) in one-half the time (22 min) was recently achieved on a 25 × 0.46 cm LiChrosorb SI 100 (5 $\mu$m) column (Brownlee Labs., Santa Clara, Calif., U.S.A.) for HRLC analysis of V, using similar solvent systems.

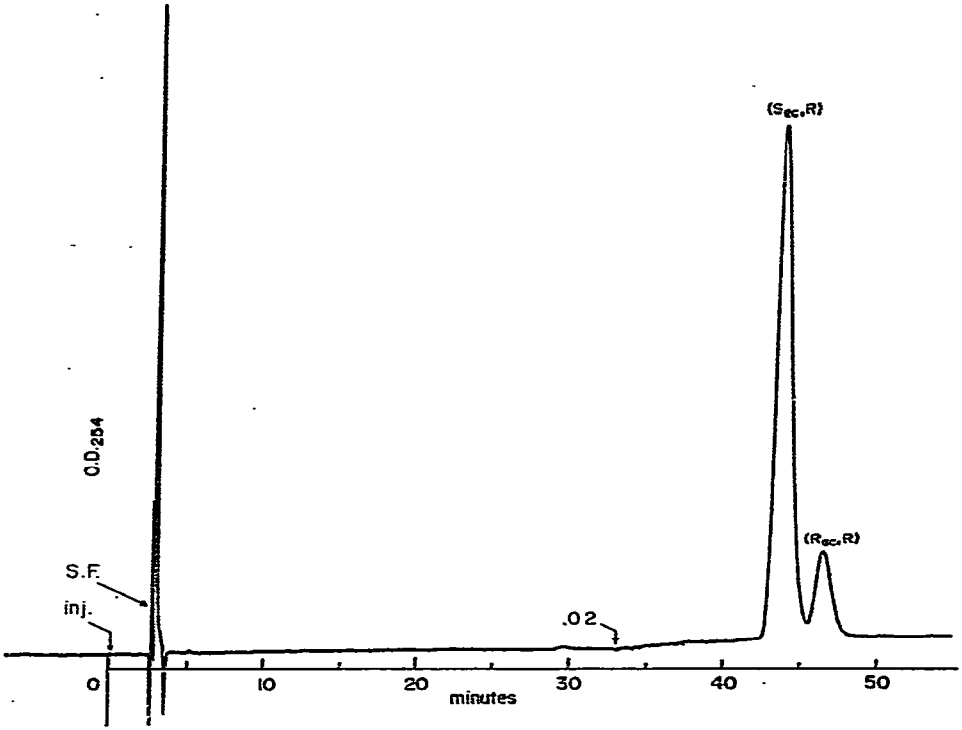



Fig. 3. HRLC analysis of diastereomeric amides V from 7-methoxycitronellic acid and (*R*)-(+)-1-(1-naphthyl)ethylamine. Conditions: Zorbax-SIL (22 × 0.46 cm I.D.) column, eluted with pentane–ethyl acetate (78:22) ("100% water saturated"), 1.3 ml/min at 300 p.s.i.g.

strong correlations between diastereomeric configuration and elution order on silica gel HRLC of a special class of carboxylic acid amides. Although using a different resolving amine and quite dissimilar chromatography conditions, we find agreement with Scott *et al.*[11] on elution patterns of different diastereomeric amide pairs, with the notable exception of the $\omega$-alkoxyl-substituted series, however. It appears that rather subtle substrate–support interactions occur, and a much wider spectrum of substituted isoprenoid acids should be analyzed before definitive rules of elution order can be established.

In conclusion, with the increased availability of high-resolution prepacked microparticulate silica gel columns and readily accessible resolving agents, rapid determinations of enantiomeric purities of selected substrates can be achieved on a routine analytical basis. Our method has improved upon existing HRLC analysis of diastereomeric citronellic and dihydrocitronellic acid derivatives, and has been extended to include 7-methoxycitronellic acid.

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