Journal of Chromatography, 174 (1979) 461–464

CHROM. 11,824

Note

Analysis of biosynthesised terpene alcohols facilitated by C_{18} phase-bonded silica

THOMAS G. McCLOUD and PETER HEINSTEIN

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Ind. 47907 (U.S.A.)

(First received September 18th, 1978; revised manuscript received February 28th, 1979)

During recent studies on the biosynthesis of terpene alcohols by a partially purified prenyl transferase (EC 2.5.1.1), we considered it desirable to combine analytical with preparative chromatography of the products. The currently available methods presented several disadvantages. Thin-layer chromatography (TLC) on silica gel containing silver nitrate will easily separate the isomers of terpene alcohols, but the relative mobilities of several C_5, C_{10} and C_{15} terpenes are so close[1–3] that use of this method for identifying a single product in a mixture containing several mono- through sesqui-terpenes would not be reliable. Consequently, preliminary classification of alcohols by chain length is necessary. Reversed-phase TLC using a silicone or mineral oil has been applied to this purpose[2,4,5], but this is both messy and time-consuming, and for further analysis, elution and removal of the silicone or mineral oil from the sample is necessary. The use of gas–liquid chromatography (GLC) proved to be unsatisfactory because recovery of injected standard [^{14}C]farnesol was consistently low (12%) and there was isomerisation on the column, as shown in Fig. 1.

High-performance liquid chromatography (HPLC) has recently been used to achieve some excellent separations of natural products[6,7]. Ross has been able to identify components of cinnamon oil by HPLC on silica[8], and of ylang-ylang and spearmint oil volatiles by reversed-phase HPLC on phase-bonded C_{18} silica[9]. Some success has been reported in separating geometric isomers by this reversed-phase technique[10]. In all these instances, the instrumentation was of the rather sophisticated and expensive type commercially available. However, many of the advantages of HPLC can be retained by performing low-pressure chromatography on phase-bonded silica in a glass column and using pumps, detectors and accessory equipment more commonly available in the laboratory.

A chromatographic column (22 × 1.2 cm) was constructed from Ace Glass No. 7 threated fittings*. The PTFE end-pieces and injection port were machined to our specifications to seal the ends of the glass column without use of additional O-rings and to be compatible with the Altex brand of fittings. During packing, a reservoir was attached to the column so that a slurry of C_{18} phase-bonded silica of

* Columns and PTFE end-fittings similar to those described are now being made available by the Ace Glass Company, Vineland, N.J., U.S.A.

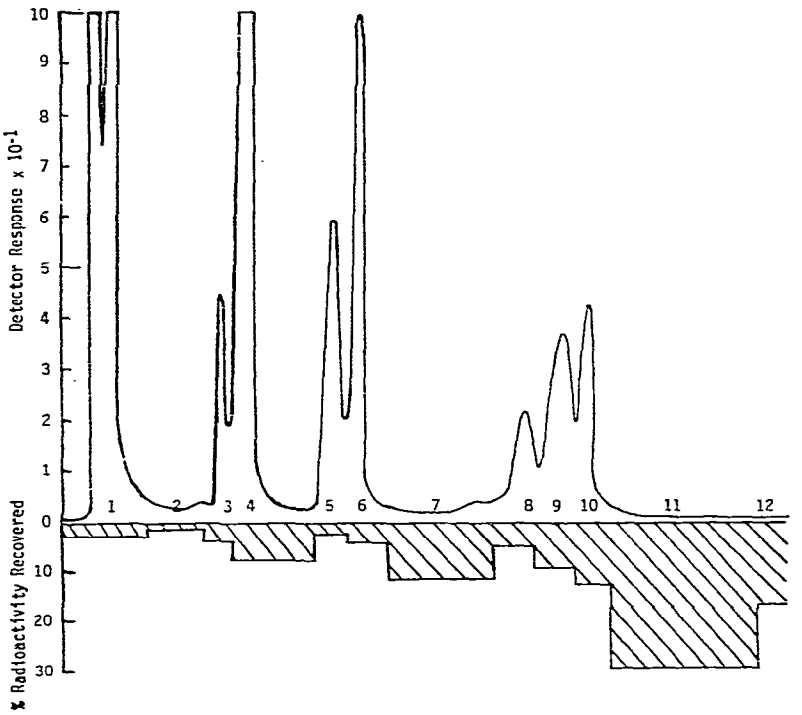

Fig. 1. Analysis of *trans,trans*-farnesol by GLC on a column packed with 10% of Carbowax on Chromosorb W (60–80 mesh) and temperature-programmed from 145° to 230° at 4°/min, with helium (0.8 ml/sec) as carrier gas and a thermal-conductivity detector. The area beneath peaks 8, 9 and 10 accounts for 26% of the total counts recovered as one of the four isomers of farnesol, whereas only 12% lies beneath the peak for *trans,trans*-farnesol; 46% of the counts recovered were eluted at times exceeding the retention time of farnesol.

the type manufactured by E. Merck (LiChroprep RP-18) could be poured in a single step and solvent under pressure could be passed through the assembly. A constant flow of solvent at 20 p.s.i. was maintained during packing, injection and elution of sample by means of a Milton-Roy high-pressure piston pump. The solvent used throughout was methanol–water–acetonitrile (7:2:1) and, during analytical runs, the flow-rate was 1 ml/min. An ISCO UA-4 ultraviolet monitor set at 254 nm was used to detect compounds as they were eluted from the column.

Samples were prepared, following incubation of various combinations of C_5 and C_{10} terpene substrates with a prenyl transferase, by hydrolysing with alkaline phosphatase, extracting with light petroleum (b.p. 30–60°), drying and concentrating[11]. To each sample was added 5 μl of isopentenol, 5 μl of nerol and 10 μl of farnesol, all from commercial sources, and the mixture was further concentrated to 50 μl, of which 20 μl were injected directly on to the column. Various standards obtained from commercial sources were also analysed in this way.

In Fig. 2 is shown a typical elution profile for an enzyme-product analysis run; the positions at which several standard compounds are eluted are indicated by arrows. As can be seen, there is excellent separation of these compounds according to the number of carbon atoms, with each group being eluted almost precisely at

the same time, regardless of their individual geometries or substituents. After elution of the last desired component, the next sample may be injected immediately, without any reconditioning of the column. Terpenoids of higher molecular weight (such as phytol and squalene) are retained on the column for extended periods, though they also may be eluted if the solvent system is made less polar.

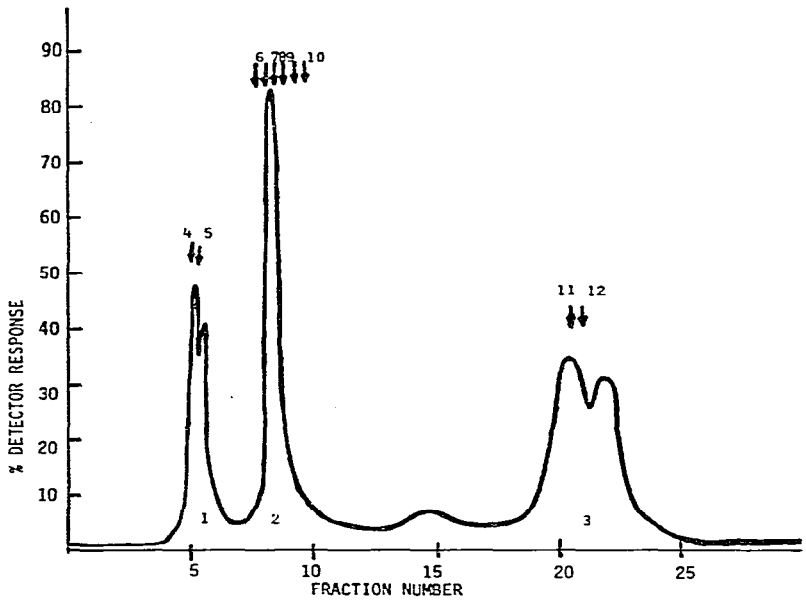

Fig. 2. Elution pattern of terpenes from phase-bonded silica. Peaks: 1 = isopentenol; 2 = nerol; 3 = farnesol (mixture of standards used in enzyme-product analysis); 4 = dimethylallylalcohol; 5 = mevalonic acid; 6 = carvone; 7 = carvacrol; 8 = geraniol; 9 = linalool; 10 = citral; 11 = bisabolol; 12 = cadinene. Fraction volume, 2.5 ml.

Fractions collected from the column were scanned for radioactivity or spotted on to a silica gel plate and sprayed with anisaldehyde reagent[12]. The peak fractions were concentrated and applied to a Merck silica gel 60 plate that had been dipped into a solution of 20% silver nitrate in acetonitrile, then briefly dried. Development was with cyclohexane–ethyl acetate (1:1), the plates were sprayed with anisaldehyde reagent, and the appropriate zones were scraped into scintillation vials for counting. Separation of the terpene alcohols by geometry has thus been obtained, and the quantitation of each individual isomer, even of such closely matched pairs as *cis,trans*- and *trans,cis*-farnesols is possible.

In addition to its uses in research, the technique presented here would seem to have great potential in industry, as it would allow for preliminary fractionation of complex mixtures, such as essential oils or fermentation products, by carbon number, thus facilitating the clean-up procedure.

ACKNOWLEDGEMENT

This investigation was supported by grant number GM 23249, awarded by the National Institute of General Medical Sciences, DHEW.

REFERENCES

1 E. Cardemil, J. R. Vicuna, A. M. Jabalquinto, and O. Cori, *Anal. Biochem.*, 59 (1974) 636.
2 E. Stahl and H. Vollman, *Talanta*, 12 (1965) 525.
3 E. Stahl and J. Jork, in E. Stahl (Editor), *Thin-Layer Chromatography*, 2nd ed., Springer, Berlin, 1969, p. 227.
4 G. P. McSweeney, *J. Chromatogr.*, 17 (1965) 183.
5 M. O. Oster and C. A. West, *Arch. Biochem. Biophys.*, 127 (1968) 112.
6 *Application Highlights No. H93 (Natural Products Isolation)*, Waters Assoc., Milford, Mass., 1978.
7 *Application Highlights No. H313 (Essential Oils)*, Waters Assoc., Milford, Mass., 1974.
8 M. S. F. Ross, *J. Chromatogr.*, 118 (1976) 273.
9 M. S. F. Ross, *J. Chromatogr.*, 160 (1978) 199.
10 J. D. Warthen, Jr., *J. Amer. Oil Chem. Soc.*, 52 (1975) 151.
11 R. Widmaier, J. Howe and P. Heinstein, *Arch. Biochem. Biophys.*, in press.
12 E. Stahl, in E. Stahl (Editor), *Thin-Layer Chromatography*, 2nd ed., Springer, Berlin, 1969, p. 857.