1. Djerassi, C., Ishikawa, M., Budzikiewicz, H., Shoolery, J. N. and Johnson, L. F. *Tetrahedron Letters* **1961** 383.
2. Dhar, M. L., Thaller, V. and Whiting, M. C. *Proc. Chem. Soc.* **1960** 310; *J. Chem. Soc.* **1964** 842.
3. Cope, A. C., Bly, R. K., Burrows, E. P., Ceder, O. J., Ciganek, E., Gillis, B. T., Porter, R. F. and Johnson, H. E. *J. Am. Chem. Soc.* **84** (1962) 2170.
4. Ceder, O., Waisvisz, J. M., van der Hoeven, M. G. and Ryhage, R. *Chimia (Aarau)* **17** (1963) 352.
5. *Varian NMR Spectra Catalog*, Varian Associates, Palo Alto, 1960, Spectrum No. 182.
6. Berkoz, B. and Djerassi, C. *Proc. Chem. Soc.* **1959** 316.

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Diphenylpicrylhydrazyl as a Reagent for Terpenes and Other Substances in Thin-Layer Chromatography

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Diphenylpicrylhydrazyl (DPPH) is a free radical of purple colour, relatively stable even in solution. It reacts with other radical species, and it has been used as a scavenger for radicals, especially for those produced in high-energy radiation. In the presence of air, DPPH reacts as a hydrogen acceptor with a variety of ethylenic compounds and phenols. The kinetics and mechanism of these reactions have been investigated by several workers.[1-4] During the reaction the purple colour changes to light yellow. This suggests DPPH as a "reversed" reagent for thin-layer chromatography. Its use as a reagent for phenols in paper chromatography has, as a matter of fact, already been suggested.[6]

DPPH was found to react with terpenes, and this note will describe its use as a sensitive visualizer for these compounds after separation by thin-layer chromatography.

Experimental. The substances to be tested have been run on silica-gypsum (13 %) thin-layer plates, dried 30 min at 110°C, and kept in an exsiccator until used. The eluating solvent was a mixture of chloroform and benzene (1:1 v/v). Approximately 100 μg of each terpene were applied to the plates. The solvent front was allowed to ascend 10 cm. After evaporation of the solvent, the plates were immediately sprayed with a solution of DPPH (Fluka, Switzerland) in chloroform (15 mg in 25 ml). The plates were then rapidly oven-heated to 110°C and kept at this temperature for 5—10 min. A positive reaction, indicating the presence of a substance reacting with DPPH, gives a yellow spot on the purple background.

Table 1.

Substances	No. of double bonds	Structure C = Cyclic AC = Acyclic	Reaction
Hydrocarbons			
Myrcene	3	AC	M
allo-Ocimene	3	AC	M
p-Cymene	3	C	W
Limonene	2	C	M
α-Phellandrene	2	C	M
β-Phellandrene	2	C	M
α-Terpinene	2	C	M
γ-Terpinene	2	C	M
Sabinene	1	C	M
α-Pinene	1	C	W
β-Pinene	1	C	W
Camphene	1	C	W
Δ^3-Carene	1	C	S
Santene	1	C	S
Fenchene	0	C	M
Humulene	3	C	S
Caryophyllene	2	C	S
Cedrene	1	C	M
Alcohols			
Linalool	2	AC	S
Lavandulol	2	AC	S
Menthol	0	C	M
4-Terpinenol	1	C	S
α-Terpineol	1	C	M
Isopulegol	1	C	S
Carveol	2	C	S
Sabinol	1	C	S
Borneol	0	C	M
Isoborneol	0	C	S
Thujylalcohol	0	C	M
Fenchylalcohol	0	C	S
neo-Isothujyl-alcohol	0	C	M

Farnesol	3	AC	S
Nerolidol	3	AC	S
Cedrol	0	C	M
Santalol	1	AC + C	S
Geraniol	2	AC	S
Citronellol	1	AC	M
Ketones			
Pulegone	1	C	M
Carvone	2	C	M
Piperitone	1	C	M
Thujone	0	C	M
Fenchone	0	C	W
Camphor	0	C	W
Ethers			
Cineole (1,8)	0	C	W
Ascaridol	0	C	S
Aldehydes			
Citronellal	1	AC	S
Hydroxy-citronellal	1	AC	S
Citral	2	AC	S
Oxides			
Limonene monoxide	2	C	S
Caryophyllene oxide	2	C	S
Esters			
Linalyl acetate	2	AC	S
Geranyl acetate	2	AC	S
Citronellyl acetate	1	AC	S
Isobornyl acetate	0	C	W
Sabinyl acetate	1	C	S
Terpinyl acetate	1	C	S
Bornyl acetate	0	C	W
Linalyl formate	2	AC	S
Other substances			
Cyclohexene	1	C	S
Heneicosene-1	1	AC	M
1,19-Eicosadiene	2	AC	M

Results. The substances specified in Table 1 have been tested. The reactivity towards DPPH is designated as strong (S), medium (M) or weak (W). The number of double bonds, and the presence of a cyclic (C) or an acyclic structure (AC), are listed for each substance.

There seems to be a definite correlation between the molecular structure and the reactivity towards DPPH. Highly unsaturated compounds with a hydroxy group, such as farnesol, exhibit a very strong reaction, whereas saturated terpene hydrocarbons show less reactivity. For terpenes with a medium reactivity, the detection limit was found to be about 1 μg with the substance being spread out on a spot of 0.5 cm in diameter.

The DPPH reaction described has been used for some time in actual analytical work on identification of terpenes and terpenoids from flowers and insects. It has proven to be a valuable alternative to $SbCl_3$ and $SbCl_5$, and the sensitivity is at least as high as that of these reagents. In some respects DPPH is to be preferred, mainly due to it being more convenient to handle than the antimony chlorides.

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1. McGowan, J. C. and Powell, T. *J. Chem. Soc.* **1961** 2160.
2. Braude, E. A., Brook, A. G. and Linstead, R. P. *J. Chem.* Soc. **1954** 3574.
3. Chapiro, A., Durup, J. and Grosmangin, O. J. *Chim. Phys.* **50** (1953) 482.
4. Chapiro, A. *J. Chim. Phys.* **51** (1954) 165.
5. Venker, P. and Herzman, H. *Naturwiss.* **47** (1960) 133.
6. Herzman, H. and Venker, P. *Z. Chem.* **1** (1961) 221.

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Correction to "Long-acting *p*-Alkoxyhydrocinnamic Acid Esters of Steroid Hormones" *

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On p. 2539 the graphs above Figs. 1 and 2 are interchanged.

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