

	Skyrin	Rubro-skyrin	Irido-skyrin	Islan-dicin
R_F	0.15	0.25	0.90	0.95
Colour	violet	green	blue violet	blue violet

The relative quantities of the different pigments were visually estimated by a direct comparison with the amounts produced by the wild type.

The culture medium was extracted with ether, the residue after evaporation redissolved in acetone, and one third of the solution used for paper chromatographic analysis. After running in a solvent consisting of chloroform-methanol-formic acid (4 %) (10:1:1) the paper was sprayed with alcoholic ferric chloride, dibromoquinone chloroimide, diazotized sulfanilic acid and phosphomolybdic acid. The culture media of the mutants, with the exception of the pigment-free ones, contained in varying amounts the aromatic compounds normally produced by the wild type.

The only difference observed between the cultures grown for three weeks and two weeks, respectively, was a higher production of the substances investigated in the former.

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A Note on Proton Magnetic Resonance Spectra of Alcohols in Carbon Tetrachloride

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In the high resolution proton magnetic resonance (PMR) spectra of the pure forms of most primary and secondary

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aliphatic alcohols, the signal from the OH-protons are obtained as multiplets. When such an alcohol, e.g. ethanol, is diluted with a nonpolar solvent such as cyclohexane or *per*-deuterocyclohexane, the OH-triplet is found to persist down to concentrations where the noise level no longer permits observations. The addition of small amounts of carbon tetrachloride to pure ethanol, however, changes the triplet into a sharp singlet¹. This is rather unexpected since carbon tetrachloride is generally assumed to be about as nonpolar as cyclohexane. We have, however, found that if commercially available carbon tetrachloride of *pro analysi* quality, which in mixtures with ethanol produces OH-singlets, was first washed with a 0.01 M solution of sodium hydroxide and then with three or four portions of water and thereafter kept away from direct or indirect sunlight, the addition of this product to a purified alcohol, e.g. ethanol, did not affect the splitting of the OH-signal, i.e. this signal was displayed as a triplet down to concentrations where the noise level no longer permitted direct observations (mole fraction alcohol ca. 0.02). In Fig. 1 the PMR spectrum at mole fraction alcohol 0.1 is shown (cf. PMR spectrum in Ref.¹, p. 3).

Exposure of a mixture of ethanol and purified carbon tetrachloride (mole fraction alcohol 0.8) to ultraviolet light or sunlight 5–30 sec. was, however, found to be sufficient to produce a sharp OH-singlet from the triplet. The extreme sen-

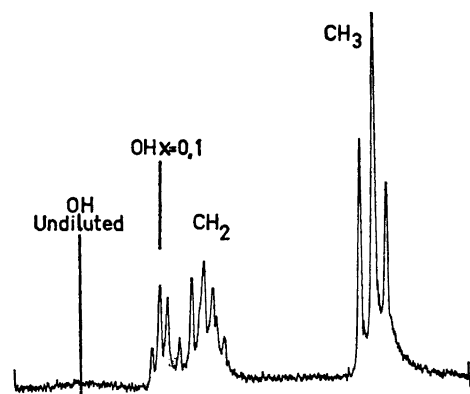


Fig. 1. PMR spectrum of ethanol in carbon tetrachloride purified according to text. Mole-fraction ethanol = 0.1. The position of the OH-triplet in pure ethanol is indicated (cf. spectrum in Ref.¹, p. 3).

sitivity towards hydrochloric acid of the shape of the OH-signal from ethanol, as shown by Arnold², would indicate that small amounts of hydrochloric acid are formed photolytically in the solution and that this is the cause of the observed alteration in the OH-signal. Further evidence for this assumption is the fact that careful addition of minute amounts of alkali to a irradiated mixture of ethanol and carbon tetrachloride brought back the OH-triplet which, upon further irradiation of the mixture, again turned to a singlet.

In addition, another observation of interest was made. When a small amount of water was added to a mixture of ethanol and carbon tetrachloride (mole fraction ethanol 0.85; water 0.05) a separate water line could be seen in the PMR spectrum to the high field side of the OH-triplet. The mixture was then irradiated for a few seconds in ultraviolet light and the PMR spectrum now revealed a complete coalescence of the water and OH-signals into one broad signal. This coalescence is normally only observed at much higher concentrations of water (mole fraction water 0.40–0.50)³. Careful addition of a small amount alkali made the coalesced line again separate into a OH-triplet and a separate water line. Repetition of the procedure revealed the coalescence and the separation to be completely reversible. The observation is clearly in line with the suggestion that hydrochloric acid accelerates the proton exchange in ethanol.²

From the observation in Ref.¹ and our own dilution experiments with commercial, unwashed carbon tetrachloride, it would appear that small amounts of hydrochloric acid are usually present in the commercial product (for an exception see Ref.⁴).

Carbon tetrachloride purified according to above should be a convenient nonpolar solvent when spin-coupling effects are to be studied in dilute solutions in which the effect of the proton exchange between the OH-groups is reduced.

The spectrometer used in this work is a Varian 4300 B NMR Spectrometer operated at 40 Mc and equipped with a V-K 3506 Flux Stabilizer.

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