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Infrared identification of lysergide (LSD)

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Infrared spectra of LSD and its tartrate salts, and factors affecting their reproducibility, have been investigated. Spectra corresponding to amorphous and crystalline forms of LSD base, the neutral tartrate and two forms of the hydrogen tartrate were obtained. The neutral tartrate was found to undergo conversion to the hydrogen tartrate on long standing. Comparison with spectra of related compounds shows that all the LSD spectra are distinctive and can be used for identification purposes, but the use of potassium bromide discs was found to cause spectral changes in the salts.

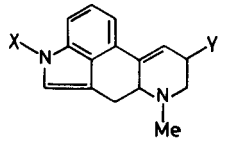
The increasingly widespread abuse of hallucinogenic drugs has led to their being controlled by legislation in many countries, which in turn has necessitated the development of simple and unambiguous methods for their identification. Attention has been concentrated mainly on lysergic acid diethylamide (lysergide; *NN*-diethyl-D-lysergamide; LSD) and its derivatives, for which methods requiring sub-milligram amounts are essential. Thin-layer or paper chromatography has been widely accepted as a means of identification, supplemented by spectrophotometry or spectrofluorimetry for quantitative estimation (Genest & Farmilo, 1964; Dal Cortivo, Broich & others, 1966; Martin & Alexander, 1967, 1968; Look, 1968). Gas chromatography has been used for the identification of other hallucinogenic amines (Genest & Hughes, 1968) but is not suitable for direct use with lysergic acid derivatives (Radecka & Nigam, 1966), although Lerner (1967) has obtained satisfactory results with the trimethylsilyl derivative of LSD. These methods, however, are not completely specific, and where evidence is liable to be contested in a court of law, a more positive identification is desirable. Mass spectrometry has been recommended for this purpose (Bellman, 1968), but is not available to all laboratories engaged in pharmacological or forensic work, and in general infrared spectrophotometry is the most widely used technique (Hale & Taylor, 1967; Cromp & Turney, 1967). It is therefore surprising to note how little attention has been paid in published methods to means of ensuring consistent spectra and to the factors affecting reproducibility.

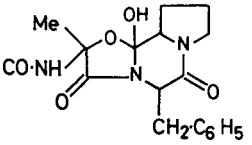
Seizures of illicit LSD may range between substantially pure samples of LSD tartrate and a variety of dosage forms including tablets, capsules, impregnated sugar cubes or blotting paper, from which the active material must be extracted, usually as the free base. Any infrared identification procedure must therefore be capable of dealing with either the salt or the base. Previous work on the infrared identification of steroids (Mesley & Johnson, 1965), sulphonamides (Mesley & Houghton, 1967) and barbiturates (Mesley & Clements, 1968) has demonstrated the necessity, whenever possible, to compare the spectrum of the sample with that of an authentic specimen recorded under the same conditions, and solvent treatments have been recommended as a means of overcoming polymorphism in these compounds. For LSD, authentic specimens are not readily available, and limitations of sample size may prevent any further treatment of the sample if the first recorded spectrum is not identifiable.

In examining samples of illicit origin it is therefore desirable to have reference spectra already available and to have ascertained in advance any factors which are likely to affect the reproducibility of such spectra. In the work here described the variability of LSD spectra has been investigated together with the spectra of some other lysergic acid derivatives with which confusion is possible.

EXPERIMENTAL

Materials

I 

II 

I. LSD	X = H	Y = $CO.NEt_2$
1-Acetyl-LSD	X = Me.CO	Y = $CO.NEt_2$
Lysergic acid	X = H	Y = CO.OH
Ergometrine	X = H	Y = $CO.NH.CH(Me).CH_2OH$
Methylergometrine	X = H	Y = $CO.NH.CH(Et).CH_2OH$
Methysergide	X = Me	Y = $CO.NH.CH(Et).CH_2OH$
Ergotamine	X = H	Y = II

Materials were obtained from the following sources: LSD (*NN*-diethyl-D-lysergamide): neutral tartrate (Delysid, LSD-25) from Sandoz Products Ltd.; synthesized sample of neutral tartrate (see discussion); also illicit samples of hydrogen tartrate and free base extracted from dosage forms. 1-Acetyl-LSD: tartrate from Sandoz Products Ltd. (ALD-52). Ergometrine (Ergonovine; *N*-[1-(hydroxymethyl)ethyl]-D-lysergamide): hydrogen maleate from Burroughs Wellcome and Co. Methylergometrine (Methylergonovine; *N*-[1-(hydroxymethyl)propyl]-D-lysergamide): hydrogen maleate from Sandoz Products Ltd. Methysergide (*N*-[1-(hydroxymethyl)-propyl]-1-methyl-D-lysergamide): hydrogen maleate from Sandoz Products Ltd. Ergotamine: tartrate from Burroughs Wellcome and Co. D-Lysergic acid: from Chemical Defence Experimental Establishment.

Spectra

Infrared absorption spectra were recorded using a Grubb Parsons GS2 grating spectrometer. Samples were prepared as mulls in Nujol (liquid paraffin) or as pressed alkali halide discs prepared from potassium bromide (Spectroscopic grade, E. Merck, A.G., Darmstadt) or potassium chloride (Hopkin and Williams Analar grade).

DISCUSSION

LSD. The reference sample of Delysid was stated by the manufacturers to be a methanol solvate of the neutral tartrate, with the formula $(C_{20}H_{25}N_3O)_2.C_4H_6O_6.CH_3OH$. Spectra of this material recorded as a Nujol mull and as a potassium bromide disc showed detail differences, but were both characterized by the absence of absorptions attributable to free carboxyl groups (Figs 1 and 2). The limited quantity available precluded investigation of the possibility of polymorphism in the neutral tartrate. The sample of synthetic material, which when first prepared gave the same spectrum as the Delysid sample, was found after two years to have undergone conversion to the hydrogen tartrate, shown by the appearance in the Nujol mull spectrum of a carbonyl absorption at 1730 cm^{-1} and by the fact that the potassium bromide disc spectrum shows bands characteristic of potassium hydrogen tartrate (Figs 3 and 4).

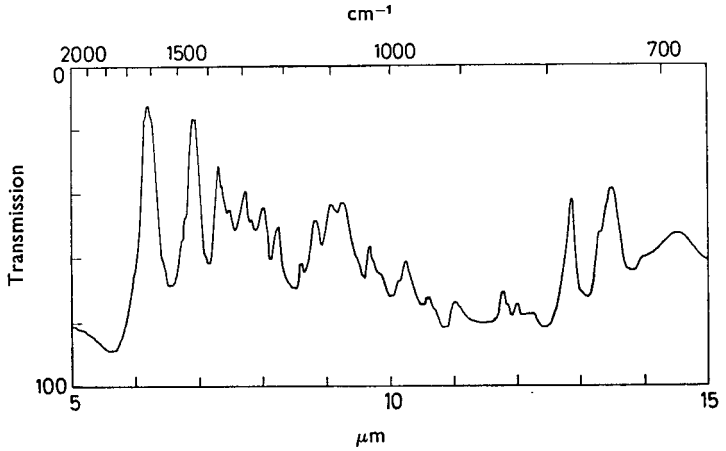


FIG. 1. Nujol mull spectrum of LSD tartrate (Delysid).

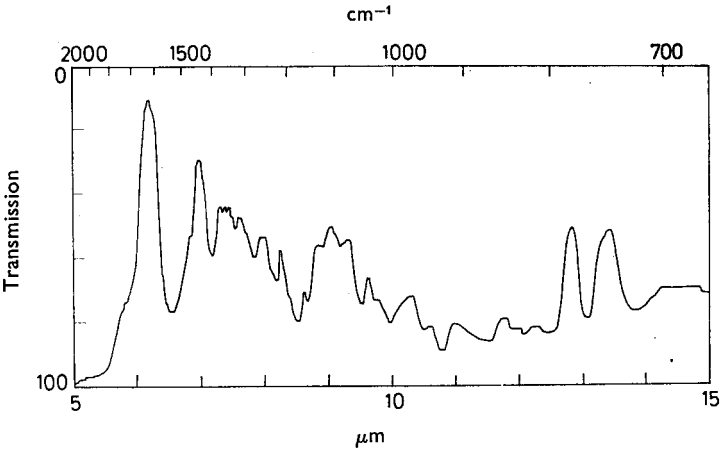


FIG. 2. Potassium bromide disc spectrum of LSD tartrate (Delysid).

A very similar potassium bromide disc spectrum (described in error as the base) appears in the Sadtler Standard Spectra (No. 30580), and evidence of partial conversion to the hydrogen tartrate has also been found in spectra from other sources of allegedly authentic LSD tartrate. It must therefore be concluded that the neutral tartrate is not stable indefinitely.

The fate of the second molecule of LSD is not clearly established, but there is some evidence to suggest that it may be present in the form of the free base. This is supported by the detection in some capsules of illicit origin of LSD in both free base and salt form. It is also uncertain whether the remaining hydrogen tartrate is still solvated with methanol. The spectrum of this material is certainly quite different from that of another illicit sample (Fig. 5) which also showed the characteristics of a hydrogen tartrate, i.e. the presence of a carbonyl absorption and conversion in a potassium bromide disc to potassium hydrogen tartrate. The latter sample was apparently unsolvated as it could be recovered unchanged from a variety of solvents. One of the patents concerning LSD (Pioch, 1956) specifically refers to crystallization of the hydrogen tartrate in the absence of methanol, and the illicit material may

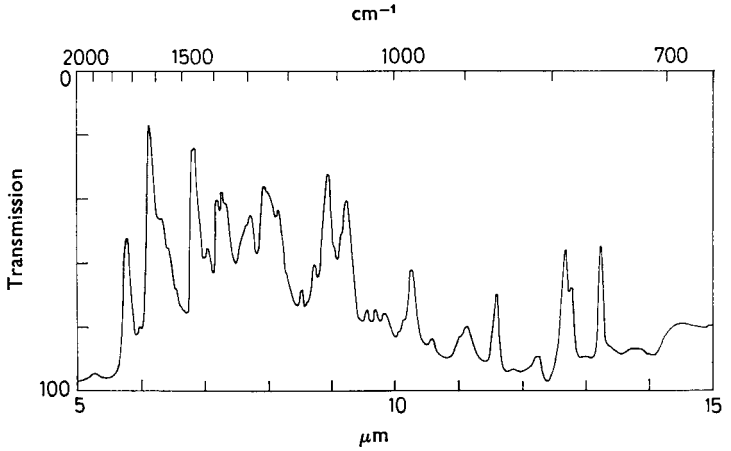


FIG. 3. Nujol mull spectrum of LSD tartrate after two years.

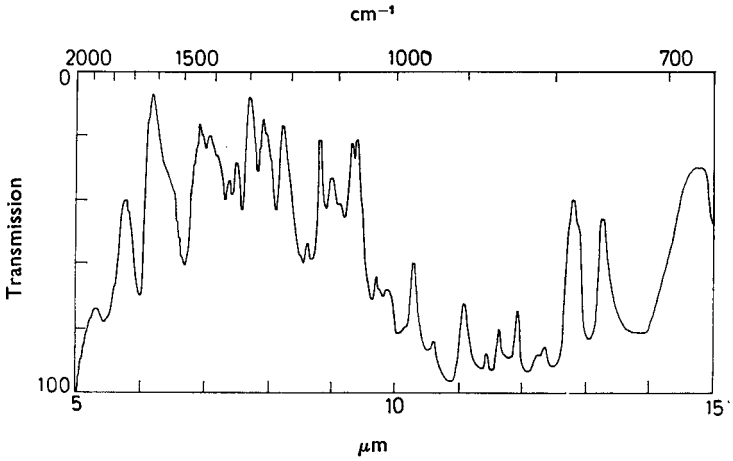


FIG. 4. Potassium bromide disc spectrum of LSD tartrate after two years.

well correspond to this product. No method of interconversion of the two forms of the hydrogen tartrate has been discovered, but both yielded LSD base when made alkaline and both showed evidence of conversion to potassium hydrogen tartrate when prepared as potassium bromide discs. The existence of two crystalline forms could thus be due either to polymorphism or to solvation of one form.

LSD base has been encountered in two forms. Evaporation of a chloroform solution directly on to a rock salt plate yielded an amorphous smear, the spectrum of which (Fig. 6) agrees with those published by Troxler & Hofmann (1957), Hayden, Brannon & Yaciw (1966), Lerner (1967) and Cromp & Turney (1967). On the other hand, precipitation of the base from an aqueous solution of the hydrogen tartrate by cautious addition of alkali gave a crystalline product, which was also obtained by crystallization of the base from aqueous acetone; a second crystalline form is also known but was not encountered in this work. The spectrum of the crystalline product (Fig. 7) is undoubtedly more distinctive than that of the amorphous form, but in practice is unlikely to be achieved from the average seizure sample of LSD, where

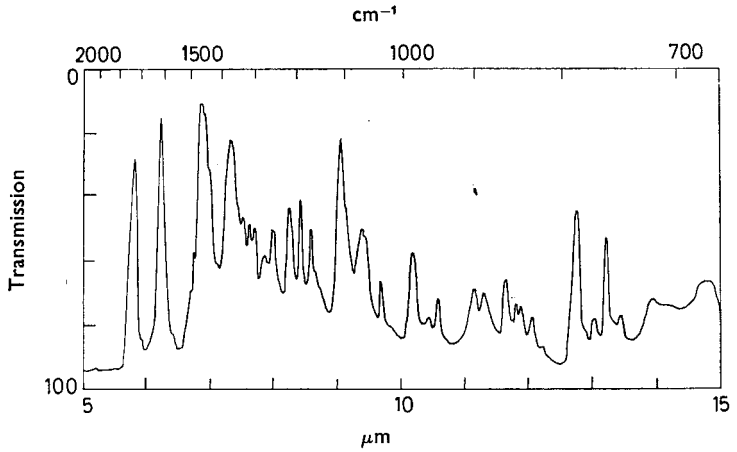


FIG. 5. Nujol mull spectrum of LSD hydrogen tartate (of illicit origin).

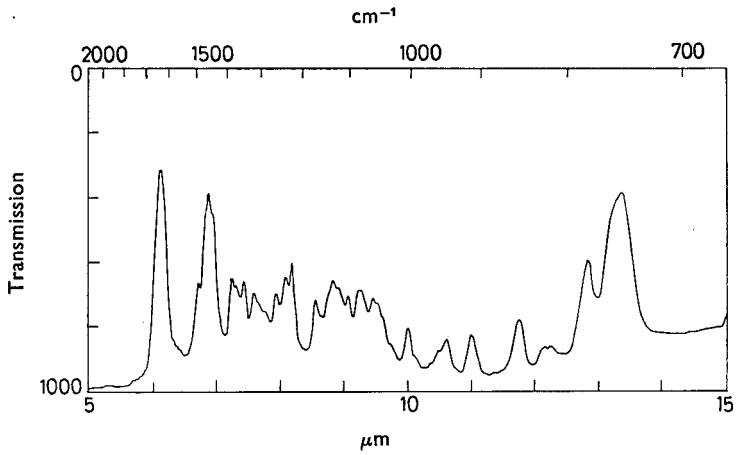


FIG. 6. Infrared spectrum of amorphous LSD base (smear from chloroform solution).

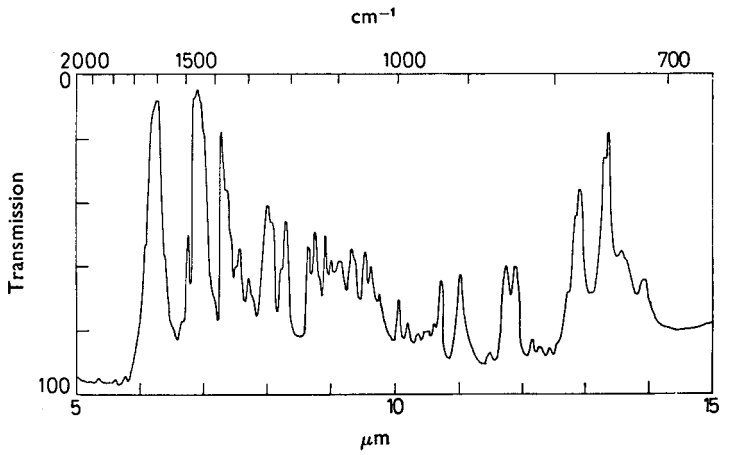


FIG. 7. Nujol mull spectrum of crystalline LSD base.

the small quantity generally precludes both recrystallization and the use of a Nujol mull. For handling sub-milligram quantities the most practicable technique is the potassium bromide disc method using cardboard or metal foil formers with a small aperture, together with a beam condenser or ordinate scale expansion, and this will normally give the spectrum of amorphous LSD.

The spectra obtained by Hale & Taylor (1967), using this technique, in fact correspond to the neutral tartrate. These workers claimed to have obtained the base from an aqueous solution of the tartrate, using sodium bicarbonate to make the solution basic before extracting with chloroform. This was presumably intended to avoid conversion to the isolysergamide derivative, but they were obviously too cautious. There appears to be no objection to the use of sodium hydroxide for this purpose, provided that the alkaline solution is immediately extracted with chloroform before epimerization can occur.

1-*Acetyl-LSD.* The small sample described as ALD-52 was a salt, identified as a hydrogen tartrate by the similarity of its infrared spectrum with that of LSD hydrogen tartrate. As with the latter the potassium bromide disc showed spectral differences from the Nujol mull consistent with ion exchange. The free base was isolated and examined as an amorphous smear deposited on a rock salt plate, giving a spectrum which agreed with that published by Troxler & Hofmann (1957) for a Nujol mull.

Other lysergamide derivatives. Ergotamine was obtained as the neutral tartrate and converted to the free base. The salt and the base had rather similar spectra, but were both readily distinguishable from all the other compounds examined. Ergometrine, methylergometrine and methysergide were all obtained as hydrogen maleates, the spectra of which, as Nujol mulls, were similar to each other but readily distinguishable. When prepared as potassium bromide discs, however, all three underwent ion exchange to give potassium hydrogen maleate and the hydrobromides of the bases. Under these conditions the methylergometrine and methysergide salts were not clearly distinguishable. The free bases all showed a tendency to darken on standing, and recovery of ergometrine and methylergometrine from a variety of solvents yielded either amorphous products or solvated crystalline forms from which the solvent could not be removed without decomposition.

Lysergic acid. The original material was probably a hydrate; recoveries from solvents yielded two additional crystalline forms and an amorphous form. A reproducible product was usually obtained by evaporation of acetone or chloroform solution on a water bath. Acidification of an aqueous solution with hydrochloric acid, followed by concentration at room temperature, yielded the hydrochloride which separated out as lustrous plates. Attempts to recover this material from organic solvents gave amorphous products which were brilliantly coloured, red from acetone, green from ethanol and sky blue from chloroform.

DISCUSSION OF INFRARED SPECTRA

All the lysergic acid amides, when examined as free bases, show prominent absorption bands near 775 cm^{-1} (medium intensity) and 745 cm^{-1} (strong), which may be ascribed to C—H out-of-plane deformation vibrations in the indole part of the molecule. The tertiary amides (LSD and 1-acetyl-LSD) have a single strong C=O band at 1600–1630 cm^{-1}; in addition, acetyl-LSD has a second band at 1700 cm^{-1},

the high frequency of which was attributed by Troxler & Hofmann (1957) to the lack of basic character of the indole nitrogen, causing the group to behave like a ketone rather than an amide. The four secondary amides examined all show the characteristic amide I and II bands at about 1640 and 1550 cm^{-1}; ergometrine, methylergometrine and methysergide also have a strong band in the 1030–1050 cm^{-1} region due to the primary hydroxyl group, and the two former compounds are distinguished from methysergide by the presence of a strong band of uncertain origin at 1200–1220 cm^{-1}. The presence of the bands at 1550 and 1040 cm^{-1} in these three compounds and at 1540 and 1725 cm^{-1} in ergotamine serves to distinguish these lysergamide derivatives from LSD.

The high frequency region is not particularly helpful, as amorphous LSD and the secondary amides all have bands centred at about 3270 cm^{-1}, which must include both N—H and O—H stretching absorptions; however, acetyl-LSD is noteworthy as having no absorptions above 3100 cm^{-1}.

The spectra of the salts show features characteristic of the anion as well as those due to the base, and as some of these are unusual they are mentioned here. All carboxylic acid salts normally show two absorptions due to the carboxylate ion at approximately 1570 and 1400 cm^{-1}. With a dicarboxylic acid in which only one hydrogen atom has been replaced, the free carboxyl group should give rise to a C=O stretching absorption near 1710 cm^{-1}. The spectra of the two forms of LSD hydrogen tartrate (Figs 3 and 5) both show a sharp absorption near 1730 cm^{-1}, the rather high frequency of which may be due to the influence of the α-hydroxyl group. Fig. 3 also shows absorptions at 1600 and 1418 cm^{-1} attributable to the carboxylate ion, but these are obscured by other bands in the spectrum of Fig. 5. The potassium bromide disc spectrum (Fig. 4), corresponding to the formation of potassium hydrogen tartrate, has bands at 1570 and 1410 cm^{-1} due to the carboxylate ion, but the 1730 cm^{-1} band is broadened and reduced in intensity and is accompanied by a weaker broad band at about 1860 cm^{-1}; the latter may be an indication of strong hydrogen bond formation in the anion.

Other tartrate absorptions occur at approximately 1105, 1075 and 690 cm^{-1} in the neutral LSD salt (the latter band being very broad), shifting to 1110, 1070 and 680 cm^{-1} in the potassium bromide discs. Corresponding absorptions in the acid tartrates are at about 1255, 1110, 1075 and 690 cm^{-1} for the mulls, whilst in the potassium bromide disc the prominent bands at 1305, 1260, 1215, 1135, 1075/1067 (doublet) and 675 cm^{-1} are all associated with the hydrogen tartrate ion, the last-mentioned band being particularly intensified. All of these bands are also present in the potassium bromide disc spectrum of ALD-52, confirming that this also is a hydrogen tartrate, although the band due to the free carboxyl group is largely obscured by the C=O absorption of the 1-acetyl group.

The maleate salts, all of which are known to be hydrogen maleates, show no free carboxyl absorption apart from a weak shoulder at about 1680 cm^{-1} in the mull spectra, which disappears altogether when they are examined as potassium bromide discs. The absence of the carbonyl absorption in the spectrum of potassium hydrogen maleate has been ascribed by Cardwell, Dunitz & Orgel (1953) to the symmetry of the anion, in which the single proton is almost centrally placed between the two carboxylate groups. Other characteristic bands in the maleates occur at 887 and 866 cm^{-1} in the mull spectra, converging in the potassium bromide discs to give a double peak at 873 and 862 cm^{-1}.

The spectra of the various forms of lysergic acid all show absorptions at approximately 1580 and 1360 cm^{-1} attributable to COO^- ions and at 2300 cm^{-1} due to NH^+, indicating internal ionization. On conversion to the hydrochloride the 2300 cm^{-1} band remains, but the carboxylate absorptions are replaced by a band at 1700 cm^{-1} consistent with the free carboxyl group.

CONCLUSIONS

The infrared spectra of LSD base and its tartrate salts are quite distinctive and can therefore be used for identification purposes. With the salts, the use of alkali halide discs can cause marked changes in the spectrum, and in the case of the maleates of ergometrine and related compounds can prevent identification of the parent base. This could conceivably also occur with tartrates of compounds closely related to LSD, so it would be wise to record a mull spectrum if quantities permit. Similarly, the mull spectrum of crystalline LSD is more distinctive than that of the potassium bromide disc, in which partial conversion to the amorphous form takes place. Nevertheless, in dealing with sub-milligram quantities of extracted bases, the pressed disc method is likely to be the only practicable way of obtaining a spectrum.

For the identification of hallucinogens infrared spectroscopy will most frequently be used in conjunction with thin-layer chromatography, and in many instances it may be necessary to use the same material for both purposes. Nothing has been said here concerning the practical technique of recovering material from thin-layer plates for infrared examination, as this has been adequately covered elsewhere (e.g., Cromp & Turney, 1967; Hale & Taylor, 1967). However, it should be emphasized that both the substrates and the solvents generally used contain impurities which may well obscure the sample spectrum when working at the 50 μg level or below. It is therefore essential to use the highest purity solvents (preferably redistilled), to wash the substrate with the eluting solvent before running the chromatogram, and whenever possible to take an authentic sample through the same procedure and use its spectrum for reference purposes.

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