Satisfactory methods for separation and purification of the different corticosteroids have been developed during the last decade. The identification of the isolated steroid, however, presents a more difficult problem. The conventional chemical evaluation of the structure is an extremely tedious task and only a few experts are able to do this with material of less than a milligram. It is impossible to use such methods in routine steroid identification. Different colour reactions have been developed for this purpose but to get unequivocal proof of the identity of a steroid many such colour reactions have to be performed. The great difficulty is that there is seldom enough material available for any extensive investigations on isolated corticosteroids. A serious drawback of the colour reactions is moreover that they destroy the amount of steroid under investigation.

In such situations the infrared spectrographic technique is very useful. The infrared spectrogram is considered as a highly specific property of a substance and during the last eight years it has been used to great advantage for steroid identification.

Infrared spectrograms can be used for quantitative determinations but they are mostly used to get qualitative information. Usually the analysis consists of running the spectrum and then comparing it with reference spectrograms that have been registered from known compounds. For most of the usually encountered steroids there are a lot of spectrograms already published chiefly from the extensive work by Dobriner and his coworkers at the Sloan-Kettering Institute in New York (1953). If for a certain substance, no published spectrogram is available, it is necessary to get an authentic sample of the substance and run the spectrum. If an isolated steroid is unknown and proper reference spectra are not available, an infrared spectrogram of this steroid can scarcely provide an irrefutable identification. However, the spectrum gives a lot of information about the chemical constitution of the steroid (Jones & Dobriner, 1949).
It is essential to make clear that it is not yet possible to tell from the infrared spectrogram, whether the substance analyzed is a steroid or not. If the steroidal nature of the compound is confirmed by other means, the infrared spectrogram can provide the following information.

1. The presence of hydroxyl groups is revealed by an absorption at about 3.0 μ. A shift of this absorption to longer wavelengths is observed if hydrogen bonding occurs in the molecule.

2. The carbon-hydrogen stretching vibrations in methyl and methylene groups give strong absorptions in the region 3.5-4.0 μ. Sometimes useful information can be obtained from a careful study of these absorptions e. g. when a double bond is present. Bending vibrations of the same groups show absorptions between 6.5-7.5 μ, which demonstrate the structural relationships of these groups in the molecule.

3. Carbonyl groups cause strong absorption bands in the region from 5.5-6.0 μ. Thorough investigation of this region can reveal much about the constitution of the steroid. For details of the many structural correlations that have been discovered, the reader is referred to the works by Jones and Dobriner and coworkers (Jones & Dobriner, 1949, Jones et al., 1949, Jones et al., 1950, Jones et al., 1952). One of the most important of these correlations is that the carbonyl absorption maximum is different for different positions of the carbonyl group in the steroid. In a steroid with several not too closely situated carbonyl groups they retain their infrared absorption characteristics and thus give several absorption peaks. Existence of hydroxyl groups can give rise to certain interaction effects which are of special importance in the determination of the structure of the side chain in corticosteroids (Jones et al., 1952). The distribution of oxygen functions and the sterical positions of the hydroxyl groups can often be elucidated from the absorption pattern in this region.

4. Steroid ester carbonyl groups in addition to an absorption in the keto group region give a strong absorption at about 8.05 μ, which makes it possible to detect steroid esters.

5. The appearance of the ester carbonyl absorption band can give information about the sterical relationship between positions 3 and 5 (Jones et al., 1951).

6. In the so-called fingerprint region from 7 to 12 μ the infrared spectrogram is dependent on the vibrations caused by the steroid as a whole. Only few specific correlations have as yet been made between molecular structure and infrared absorption in this region, where the absorption seems specific for each steroid. The survey of spectra from over one thousand steroids at the Sloan-Kettering Institute in New York has failed to detect two steroids with the same infrared spectrogram (Dobriner et al., 1953).

The easiest method of obtaining the infrared spectrum of a substance is perhaps to analyze it in solution. Unfortunately every possible solvent has its own
infrared spectrum that obscures some part of the spectrum of the solute. The best and most used solvent is carbon disulphide, which is completely transparent for infrared radiation in the hydroxyl group and carbonyl group regions and for most of the fingerprint region even in layers of several millimeters. The carbon-hydrogen stretching and bending vibrations must be studied in carbon tetrachloride solution. The most serious drawback with carbon disulphide is its poor solvent capacity for polar compounds. Unfortunately, most corticosteroids belong to this class of substances and cannot be analyzed in carbon disulphide solution. Chloroform is a better solvent for corticosteroids but has its own strong absorption bands that permit only glimpses of the spectrum except for those steroids that are very soluble and therefore can be analyzed in thin layers. The use of a double beam infrared spectrograph facilitates the use of solvents with strong absorption bands. Acetylation of the corticosteroids makes them more soluble in the abovementioned solvents and can sometimes be used.

Most corticosteroids, however, must be analyzed in a solid state and for that purpose several techniques exist. Before they are discussed something must be said about the amount of substance that is necessary to obtain a satisfactory infrared spectrogram. For substances in solution and with suitable microcells amounts of about 100 µg. will give a good spectrogram. For substances that must be analyzed as solids, amounts ten times greater or more have been necessary. Several successful attempts to construct infrared microscopes have therefore been made (Blout et al., 1950, Cole & Jones, 1952, Anderson & Woodall., 1953, Coates et al., 1953). The effect of an infrared microscope is to condense the light path in the spectrograph to a very small beam in which the sample can be positioned. My own experience has been made with a Perkin-Elmer infrared microscope attached to a spectrophotometer model 12-C (Coates et al., 1953).

The technique for analysis of solids in the infrared microscope is essentially the same as is used for macro samples. The one most used up to the present is the nujol mull. The substance to be analyzed is ground in a small agate mortar together with nujol (paraffin oil) to a paste that is squeezed between two sodium chloride plates and is thus analyzed. In the microscope one plate is sufficient on which the suspension of the steroid is placed as it is analyzed in a horizontal position. Excellent spectra are obtained with this technique. The disadvantages are the absorption bands of the paraffin oil and the need of using comparatively large quantities of substance. It is difficult to mull smaller amounts than 50–100 µg. As the area used in the microscope for the recording is of the order 50 times 100 microns the spectrum will actually be run on a much smaller amount of substance, usually not more than 5 µg.

In 1952 a new technique for analysis of solids by infrared spectrography was published (Schi ed t & Reinwein, 1952. Stimson & O'Donnell, 1952). The substance to be analyzed is mixed with potassium bromide powder and pressed
to a plate in a vacuum. The plate that is glass clear, is placed in the spectrograph and the spectrum recorded. Anderson & Woodall have used this technique in their infrared microscope (1953) with very good results. It is probable that it will supersede the nujol mull technique.

A substance can also be melted on a sodium chloride plate or deposited there from a solution. A single crystal can also be analyzed in the microscope and if polarized infrared radiation is used information can be obtained about the steric structure of the substance. The deposition of substance from a solution can be used when very small amounts are available. It is essential, however, to keep the evaporation area as small as possible. To achieve this I have dissolved a steroid in the smallest possible volume of chloroform and then dipped a capillary in the solution, whereby the solution sucks into the capillary by the capillary force. The tip of the capillary is then placed in contact with the sodium chloride plate. In order not to scratch the plate the capillary is made from polyethylene. The solution runs out of the capillary as fast as the solvent evaporates, and thus the steroid is deposited in a ring, the diameter of which is equal to the diameter of the capillary. In Fig. 1 is shown the spectrogram of desoxycorticosterone acetate (about 5 µg.) obtained from a sample treated by this technique. The curve is the instrumental curve as obtained from the instrument without any corrections.

As a rule the losses of substance by manipulation are considerable and in

![IR microscope spectrogram of desoxycorticosterone acetate obtained with infrared microscope.](image-url)
order to get a spectrum of one microgram of substance I should estimate that about ten micrograms are needed.

A most special technique has been worked out by Blout et al. (1952) for analysis of substances in solution in the microscope. The solution is enclosed in a capillary made of silver chloride and the ends of the capillary are sealed. All operations are made with the aid of a micromanipulator. The cells are used only once and stored for reference.

Improvements in the handling techniques will undoubtedly be made but it is wise to remember as Blout has pointed out (1952) that »... although it is now possible to obtain satisfactory infrared spectral data on about one gamma of organic compound in solution, several times that amount of substance usually is necessary because of manipulative losses incurred in handling and transferring the materials«. But even so the advantages of these new developments in the infrared field are obvious. Many problems in the field of steroid synthesis and metabolism within the human body, that await their solution, will be greatly aided by infrared spectrography.

**SUMMARY**

The possibilities and advantages of infrared spectrography as an analytical tool for investigations of corticosteroids are discussed. The methods for obtaining the infrared spectrogram with special reference to the use of an infrared microscope are surveyed. A convenient method of depositing small amounts of substance on sodium chloride plates is described, whereby it has been possible to obtain spectrograms from as little as ten micrograms of a steroid.

**REFERENCES**


355