TABLE III.—ASSAY OF COMMERCIAL INJECTIONS

| Product | pH | Chlorobutanol Claim, % | Chlorobutanol Found, % | Chlorobutanol % Recovery of Added[a] |
|---|---|---|---|---|
| Thiamine HCl, 100 mg./ml. | 2.61 | 0.35 | 0.27 | 96.0 |
| Isoniazid, 100 mg./ml. | 6.01 | 0.50 | 0.47 | 98.3 |
| Oxytocin, 10 units/ml. | 3.52 | 0.50 | 0.56 | ... |

[a] See text.

dilution of 132 mcg./ml. The results are presented in Table II.

**Adherence to Beer's Law.**—Beer's law was followed strictly in the concentration range studied. A typical Beer's law plot is presented in Fig. 1, where each point is the average of at least duplicate samples.

**Assay of Chlorobutanol in Commercial Injections.**—The standard procedure described above was used to estimate chlorobutanol in thiamine hydrochloride, isoniazid, and oxytocin vials. These were products of indeterminate age from hospital stocks. There was considerable variance noted in the declared and found concentrations of preservative in the preparations as shown in Table III. The assays were validated by recovery experiments in which known amounts of preservative were added to two of the commercial preparations. The amount recovered was calculated from assay values with and without added chlorobutanol. All data represent the average of duplicate or triplicate assays.


## SUMMARY

A procedure for estimating chlorobutanol in parenteral dosage forms is described. The procedure requires only commonly available laboratory equipment, and it is rapid and selective. The sensitivity is sufficient for use in assay of unit doses. The method appears to be accurate to about ±5%.


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# Infrared Identification of Indole Ring in Indoles and Indole Alkaloids in the 700 to 400 cm.$^{-1}$ Region

By R. J. WARREN, I. B. EISDORFER, W. E. THOMPSON, and J. E. ZAREMBO


**The spectra of 23 indoles of varying structure have been recorded and analyzed in the range of 700 to 400 cm.$^{-1}$. All of the indoles studied have two characteristic absorption bands in this region. The first is located at 620 ± 20 cm.$^{-1}$ and the second at 575 ± 25 cm.$^{-1}$. Spectra-structure correlations of the absorption bands are presented and discussed. These bands appear to be characteristic of the indole moiety and should be useful for verification of suspected indole structures.**


THE AVAILABILITY of commercial instrumentation extending into the far infrared region has heightened interest in this relatively unexplored area. As various functional groups and classes of compounds are investigated, the region gains in importance. Previous publications have shown that certain functional groups and ring systems have characteristic absorptions in this area. Aldehydes (1), ketones (2), amides (3), and aliphatic and aromatic hydrocarbons (4) are among those shown to possess unique absorptions in the far infrared. With few exceptions, however, there has been little reported on heterocyclic systems. This paper reports the results of an infrared study of the biologically important indole ring system. Twenty-three indoles of varying structure have been studied in the 700 to 400 cm.$^{-1}$ region, and all show two characteristic bands in this range. This study provides definitive information on the presence of the indole ring, a feature which is not available in the 4000 to 650-cm.$^{-1}$ region.


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The authors are grateful to Mrs. Eleanor Cherry and Mr. Peter Begosh for their technical assistance in obtaining the infrared spectra and to Dr. Jerry Weisbach for samples of 4-methoxyindole and the indole alkaloids presented here.


## EXPERIMENTAL

With the exception of 4-methoxyindole, which was synthesized at these laboratories, all of the indoles studied were commercially available.

The spectra were recorded on a Perkin-Elmer model 521 spectrometer. The samples were prepared and run as mineral oil mulls on KBr plates in the case of solids and as natural films in the case of liquids.

## RESULTS AND DISCUSSION

Table I lists the indoles studied and the major absorption bands found to be characteristic for the series. It can be readily seen that the range of correlation frequencies is a narrow one and varies by ±20 cm.$^{-1}$ for the first absorption and ±25 cm.$^{-1}$

TABLE I.—INFRARED ABSORPTION FREQUENCIES OF INDOLE COMPOUNDS

| Indole | 609 cm.$^{-1}$ | 587 cm.$^{-1}$ |
|---|---|---|
| *N*-Methylindole | 608 | 572 |
| 2-Methylindole | 609 | 575 |
| 3-Methylindole | 610 | 572 |
| 5-Methylindole | 618 | 589 |
| 7-Methylindole | 617 | 575 |
| 4-Methoxyindole | 620, 635 | 600 |
| 5-Methoxyindole | 615 | 599 |
| 7-Methoxyindole | 615, 605 | 594 |
| 4-Methoxytryptamine | 611 | 589 |
| 6-Methoxytryptamine | 610 | 593 |
| 7-Methoxytryptamine | 605, 615 | 572 |
| Ibogaine | 621, 631 | 575 |
| Harmane | 634 | 569, 581 |
| Ajmalicine | 606, 618 | 587 |
| Tetraphyllicine | 603, 627, 637 | 541, 572, 591 |
| Tetraphylline | 625, 637 | 581 |
| Ibogamine | 602 | 550, 581 |
| Bufotenine | 606, 644 | 594 |
| Gramine | 635 | 572, 587 |
| 3-Formylindole | 638 | 597, 537 |
| Harmine | 598, 637 | 550 |
| 3-Indoleacetic acid | 598, 622 | 581 |

for the second. It would seem apparent then that these two absorptions could be used as a diagnostic tool to determine the presence or absence of the indole system.

**620 cm.$^{-1}$ Region.**—All indoles have at least one band in this region. In some cases more than one band is observed, but this may be due to splitting of a single broad band. The consistency and persistence of the occurrence of this absorption throughout the series permits assignment of the absorption to the indole ring system. This assignment is also in agreement with previously published Raman data (5). The spectral data presented here further suggest that this absorption is due to that portion of the indole ring not containing the nitrogen atom. If we examine the data reported for the methyl- and methoxyindole series, we find that the frequency of the 620 cm.$^{-1}$ band increases relative to indole itself when the substituent is in the 4, 5, 6, or 7 position. Presence of the substituent in the 2 or 3 position has no perceptible effect on the frequency. This would indicate the further possibility and utility of this band as an indicator of the substitution position of indoles.

**575 cm.$^{-1}$ Region.**—The second characteristic absorption of this series of compounds is located at 575 ± 25 cm.$^{-1}$, and this is also assigned to the indole ring system. This assignment is also in agreement with previously published Raman data (6). This absorption, like the one at 620 cm.$^{-1}$, is remarkably consistent and found in every member of the series. The nature of the substituent on the ring appears to have little effect on the position or intensity of the two absorptions. The position of the substituent does involve small shifts in the frequency of the absorption but, other than that, these two absorptions are independent of the type of substituent.

SUMMARY

Data have been presented to show that indole ring compounds possess two characteristic absorptions in the range 700–400 cm.$^{-1}$. The absorptions are consistent in location and independent of the substituent on the ring.

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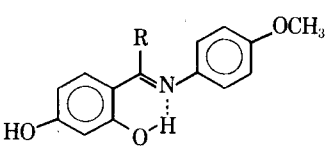
# Synthesis and Pharmacology of Some Azomethines

By E. O. MAGARIAN*, ALTA RAY GAULT, and W. LEWIS NOBLES

**Three azomethines were prepared and evaluated for estrogenic activity according to the Allen-Doisy method. They demonstrated weak estrus exciting properties.**

THE THERAPEUTIC usefulness of the estrogens in the treatment of prostatic and breast carcinomas is well known (1). Hormones are essential for the growth and functional development of certain normal tissues, and there seems to be little doubt that these hormones may also be involved in the development of malignancies of these tissues. Perhaps the hormonal imbalances induced by the administration of estrogens result in the temporary regression of these tumors.

Several azomethines have been prepared and tested for estrogenic activity (2). Nomura (3) prepared and evaluated compounds of the following type:

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* Present address: Department of Pharmaceutical Chemistry, College of Pharmacy, University of Rhode Island, Kingston.