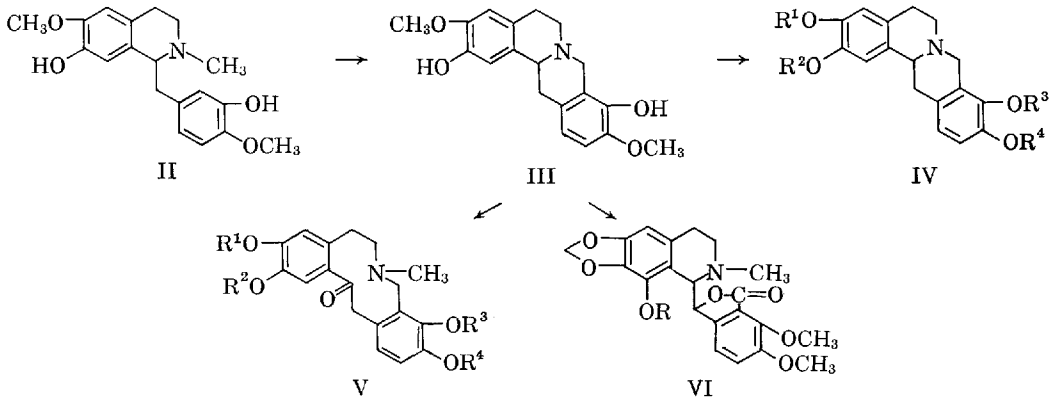

Scheme II

α-allocryptopine in opium appears to be of the order of 0.01%.

Anal.[5]—Calcd. for $C_{21}H_{23}NO_5$: C, 68.28; H, 6.28; N, 3.79. Found: C, 68.24; H, 6.03; N, 3.99.

DISCUSSION

Reticuline (II) has been shown to be a precursor for protopine in several plant species (11, 12), and there is evidence that this biotransformation proceeds by way of scoulerine (III) (12), which has recently been isolated from opium (6). Scoulerine, like reticuline, represents an important branching point in the biosynthesis of opium alkaloids from which a number of tetrahydroprotoberberine (IV), protopine (V), and phthalideisoquinoline alkaloids (VI) may be derived (Scheme II). A thorough search may well reveal other members of these alkaloid groups in the opium poppy than those which have been reported so far.

[5] The analyses were carried out by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

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Communications

Nonspecificity of Published Assays for Chloramphenicol Solutions

Sir:

The authors have found that the accepted spectrophotometric method (1) for chloramphenicol-containing pharmaceuticals does not yield valid results when applied to a partially degraded aqueous solution of chloramphenicol. The Code of Federal Regulations designates as acceptable various analytical methods for the antibiotic in pharmaceuticals. The procedures for two microbiological methods and one spectrophotometric method are outlined in the code (1). In addition, Higuchi, Marcus, and Bias have developed a different microbiological method and have compared this with a chromatographic method for chloramphenicol (2).

Samples of an aqueous solution of chloramphenicol containing stabilizing agents were stored at 4°, 22°, and 32° for approximately 16 months. Table I shows the chloramphenicol content of

TABLE I.—COMPARISON OF METHODS[a]

Storage Temp.	Microbiological (Cup Plate)	Microbiological (Turbidimetric)	U. V. with Prior TLC Separation	Direct U. V.
Std.[b]	0.492 ± 0.011	0.507 ± 0.001	0.491 ± 0.003	0.505 ± 0.002
4°	0.517 ± 0.005	0.515 ± 0.001	0.497 ± 0.005	0.555 ± 0.000
22°	0.390 ± 0.013	0.390 ± 0.007	0.383 ± 0.001	0.556 ± 0.002
32°	Below 0.240	Not assayed	0.183 ± 0.002	0.566 ± 0.004

[a] Each figure represents the average of three determinations and is expressed as the per cent of chloramphenicol in the solution. The precision is expressed as average deviation from the values given. [b] Freshly prepared solution of the same formulation containing 0.50% chloramphenicol.

TABLE II.—ANALYSIS OF COMMERCIALLY AVAILABLE CHLORAMPHENICOL PRODUCTS[a]

	U. V. with Prior TLC Separation	Direct U. V.
Product *1*	0.189	0.242
Product *2*	0.166	0.221

[a] Each figure represents the average of two determinations and is expressed as the per cent of chloramphenicol in the solution. The label claim on these two products was 0.2% chloramphenicol.

these samples after 16 months as determined by two accepted microbiological methods (1), by the accepted ultraviolet method (1), and by an improved spectrophotometric method utilizing thin-layer chromatography. The solution was originally prepared to contain 0.55% chloramphenicol.

The improved spectrophotometric method consisted of quantitative separation of chloramphenicol from degradation products utilizing thin-layer chromatography followed by ultraviolet analysis. Standard 2 by 9 in. thin-layer chromatography plates were coated with silica gel $HF_{254 + 366}$ (Brinkmann Instruments, Inc.) and preconditioned before use by heating in an oven at 90° for 15 min. The developing medium was chloroform–isopropanol (4:1) and visualization of the antibiotic was accomplished using an ultraviolet light emitting at 2537 Å. Development time for the chromatogram was 55 min. The chloramphenicol was desorbed from the adsorbent by ethanol over a 12-hr. period and determined at 274 mμ on an ultraviolet spectrophotometer.

It can be seen from the data in Table I that all four methods of analysis agree closely on a freshly prepared aqueous solution of chloramphenicol. It is also evident from the above table that direct ultraviolet analysis of a partially degraded aqueous solution of chloramphenicol which is 16 months old yields high values. These figures are not in agreement with the two accepted microbiological methods which are specific for active antibiotic concentration. On the other hand, the results using prior separation by thin-layer chromatography followed by ultraviolet determination are in close agreement with those values obtained by the microbiological methods. In fact, if the 0.183 figure obtained by the thin-layer chromatography method on a 32° sample is used, it can be calculated that the chloramphenicol in this sample has undergone approximately 67% degradation. A determination by direct ultraviolet analysis on the same sample shows that no degradation has taken place.

The thin-layer chromatographic method and the accepted direct ultraviolet method (1) were applied to stable aqueous solutions of chloramphenicol currently available only on the foreign market. The results, shown in Table II, indicate considerably less active chloramphenicol present in samples tested by the thin-layer chromatographic method than by the direct spectrophotometric method.

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