

# Colorimetric Assay of Amphotericin B

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**A**MPHOTERICIN B, like nystatin, is usually assayed by microbiological methods (1, 2). Following the development of the colorimetric assay of nystatin (3), the method was applied to amphotericin B and promising results were obtained.

The procedure of the assay was the same as for nystatin (3), except that the concentration of amphotericin B solution was about 80 mcg./ml. and absorbance measurements were made at 435 m $\mu$ . Results of assays of several samples of powder are shown in Table I.

The colorimetric assays show good reproducibility with a standard deviation of about 2%. The results agree well with the microbiological assays which have a standard deviation of about 2.7%.

It was observed that the absorbance of the color extracted into chloroform was lower if the amphotericin B solution was allowed to stand in the presence of sodium hydroxide, indicating that this method, as in the case of nystatin, may also be a stability assay for amphotericin B. Stability experiments and assays of pharmaceutical preparations are now in progress.

## REFERENCES

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TABLE I.—COLORIMETRIC ASSAY OF AMPHOTERICIN B

Sample <sup>a</sup>	Colorimetric Assay <sup>b</sup> (mcg./mg.)	Average (mcg./mg.)	Microbiological Assay <sup>c</sup> (mcg./mg.)
Powder #1	807	792	801
	809		
	772		
Powder #2	781	821	786
	828		
	816		
Powder #3	820	765	743
	819		
	771		
Powder #4	760	863	864
	765		
	877		
Powder #5	879	878	879
	832		
	866		
Powder #6	886	797	802
	883		
	789		
Powder #7	805	915	908
	941		
	920		
Powder #8	895	788	825
	903		
	764		
	796		
	783		
	794		
	804		

<sup>a</sup> Stored at 0° and colorimetric assays performed over a 4-month period. <sup>b</sup> Each value represents an individual assay with an independent sample preparation. <sup>c</sup> Assayed by the turbidimetric method using *Candida tropicalis*.

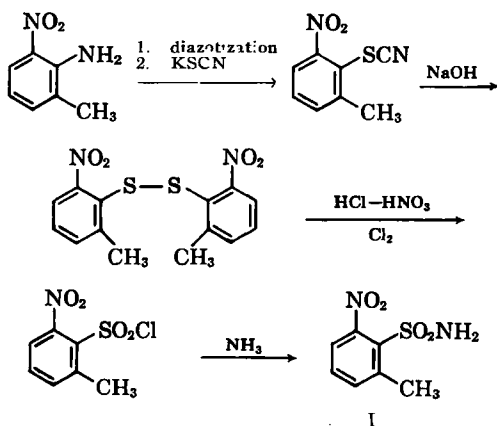
## Saccharin Derivatives VII. Synthesis of 7-Nitrosaccharin and 7-Aminosaccharin

By GLENN H. HAMOR

**R**ECENT work has shown that alkyl 4-amino-2-sulfamoylbenzoates possessing marked anti-convulsant activity may be prepared by alcoholysis of 6-nitrosaccharin, followed by reduction (1). Therefore, the preparation of 7-nitrosaccharin was undertaken as a starting material for synthesis of further compounds to be used in a structure-activity correlation study of anticonvulsants. In addition 7-aminosaccharin was synthesized in the hope that it, along with 7-nitrosaccharin, might give useful information concerning the relationship of chemical structure to taste (2).

The parent compound, 6-nitro-*o*-toluenesulfona-

mid (I), was prepared by the method of Szabo (3) according to the series of reactions shown below.



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