conditions *e.g.* during cell division or during growth in the dark[20,21]; thus paramylon can probably be classified with those 1,3-β-glucans which act as reserve substances[22,23].

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1 J. GOTTLIEB, *Ann. Chem. Pharm.*, 75 (1850) 50.
2 H. KYLIN, *Kgl. Fysiograf. Sällskap. Lund. Forh.*, 14 (1944) 221.
3 J. HABERMANN, *Ann. Chem., Liebigs*, 172 (1874) 11.
4 F. KUTSCHER, *Z. physiol. Chem., Hoppe Seyler's*, 24 (1898) 360.
5 O. BÜTSCHLI, *Arch. Protistenk.*, 7 (1906) 197.
6 S. H. HUTNER AND R. C. HOCKETT, cited by S. H. HUTNER AND L. PROVASOLI, in A. LWOFF, *Biochemistry and Physiology of Protozoa*, Vol. 1, Academic Press, New York, 1951, p. 47.
7 S. H. HUTNER, M. K. BACH AND G. I. M. ROSS, *J. Protozool.*, 3 (1956) 101.
8 M. G. SEVAG, D. B. LACKMAN AND J. SMOLENS, *J. Biol. Chem.*, 124 (1938) 425.
9 G. KLEBS, *Untersuch. Bot. Inst. Tübingen*, 1 (1883) 233.
10 A. ARNOLD, *Naturwissenschaften*, 43 (1956) 233.
11 I. MANDL AND C. NEUBERG, *Arch. Biochem. Biophys.*, 35 (1952) 326.
12 S. PEAT, W. J. WHELAN AND H. G. LAWLEY, *J. Chem. Soc.*, (1958) 727.
13 G. O. ASPINALL AND R. J. FERRIER, *Chem. and Ind.*, (1957) 1216.
14 D. R. KREGER AND B. J. D. MEEUSE, *Biochim. Biophys. Acta*, 9 (1952) 699.
15 D. J. BELL AND D. H. NORTHCOTE, *J. Chem. Soc.*, (1950) 1944.
16 S. PEAT, W. J. WHELAN AND T. E. EDWARDS, *J. Chem. Soc.*, (1958) 3862.
17 E. G. PRINGSHEIM, *Nature*, 173 (1954) 775.
18 K. P. SINGH, *Am. J. Botany*, 43 (1956) 274.
19 G. DEFLANDRE, *Bull. biol. France et Belg.*, 68 (1934) 382.
20 F. MAINX, *Arch. Protistenk.*, 60 (1927–28) 355.
21 E. G. PRINGSHEIM, *Nova Acta Leopoldina*, 125 (1956) 1.
22 B. A. STONE, *Nature*, 182 (1958) 687.
23 A. R. ARCHIBALD, D. J. MANNERS AND J. F. RYLEY, *Chem. and Ind.*, (1958) 1516.

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An improved synthesis of scopoletin

The occurrence of scopoletin (6-methoxy-7-hydroxy-coumarin) in cigarette smoke[1], and inhibition of root growth by external application of scopoletin to *Avena* and *Phleum* roots[2] have pointed out the need for a feasible synthesis to produce pure scopoletin in good yield. The present communication reports an improved synthesis of scopoletin based on modifications of the method reported by AGHORAMURTHY AND SESHADRI[3]. The pure scopoletin thus prepared has been used for preliminary studies of its metabolism in laboratory rats[4].

The new, modified procedure gives an overall yield of pure scopoletin of 55 %. In our hands, the procedure of AGHORAMURTHY AND SESHADRI gave a considerably

lower yield as large losses occurred in isolating the scopoletin in pure form from mixtures of isomers and other related products.

For the synthesis of scopoletin, 12.8 g $NaHCO_3$ and 13 g benzyl chloride were added to 500 ml of a solution of 25 g esculin (6-glucoside of 6,7-dihydroxycoumarin) in absolute ethanol, and the reaction mixture was refluxed 48 h on a steam bath. 1 l water was added to the cooled solution, and the resulting precipitate was filtered and dried, m.p. 182°. 30 g of the 7-benzylesculin were hydrolyzed, using 500 ml 7 % H_2SO_4, with heating on a steam bath for 6 h. The white precipitate that formed after cooling in a refrigerator was recrystallized by solution in methanol and addition of water until cloudiness resulted, and then again cooling in the refrigerator; m.p., 192°.

The 7-benzylesculetin (14.2 g) was dissolved in 300 ml anhydrous acetone, and 21.8 g anhydrous K_2CO_3 and 14.6 g dimethyl sulfate were then added, and the mixture was refluxed for 8 h on a steam bath. After filtration, the filtrate was evaporated to about 25 ml, and water added until the 7-benzyl-6-methyl ether of esculetin precipitated. After recrystallization from aq. methanol, its m.p. was 123°. The 7-benzyl-6-methyl ether of esculetin (13.8 g) was dissolved in 150 ml glacial acetic acid plus 75 ml conc. HCl and heated for 4 h on a steam bath. After the solvent was removed *in vacuo*, the precipitate was dissolved in methanol and decolorized with charcoal. The solution was allowed to evaporate slowly to produce crystals, m.p. 205°. The compound was identical with reference scopoletin on paper-chromatographic and infrared-absorption spectral comparisons.

When developed in *n*-butanol–ethanol–$(NH_4)_2CO_3$ buffer (40:11:19, v/v/v)[5], the paper chromatogram of this scopoletin preparation revealed the presence of trace amounts of two other blue-fluorescing compounds. To remove these impurities, the scopoletin was dissolved in the least possible amount of satd. aq. Na_2CO_3. The solution was then extracted several times with 100-ml portions of chloroform, and then slowly acidified with HCl. Fine white needles of scopoletin resulted. These did not contain any blue-fluorescing substances other than scopoletin when analyzed by paper chromatography in the buffer system just mentioned.

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[1] C. H. YANG, Y. NAKAGAWA AND S. H. WENDER, *J. Org. Chem.*, 23 (1958) 204.
[2] B. M. POLLOCK, R. H. GOODWIN AND SUSAN GREENE, *Am. J. Botany*, 41 (1954) 521.
[3] K. AGHORAMURTHY AND T. R. SESHADRI, *J. Sci. Ind. Research (India)*, 11B (1952) 411.
[4] H. D. BRAYMER, *Ph. D. Dissertation*, University of Oklahoma, 1960, p. 8–15.
[5] M. E. FEWSTER AND D. A. HALL, *Nature*, 168 (1951) 78.

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