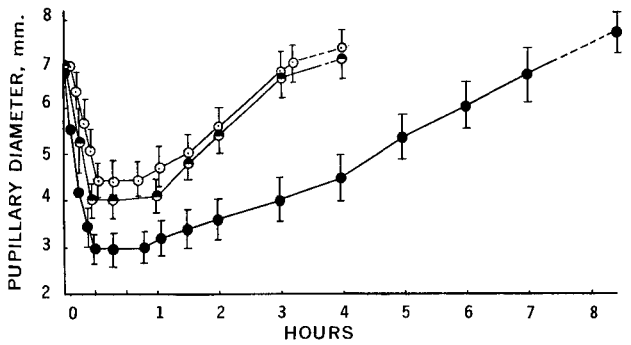


Figure 1—*Miotic responses from liquid and solid dose cul-de-sac treatments. Key: ⊙ pilocarpine alginate solution, 3.34%; ◓, pilocarpine hydrochloride, 2% (methylcellulose); ●, pilocarpine alginate flake, 4.9 mg. Broken lines signify rebound activity. Bars represent standard deviation,* N = *10–14.*

Our present findings show that the 1,4-β,D-mannuronic acid, commonly known as alginic acid, salt of pilocarpine when administered as an ophthalmic disk by this route provides a significantly greater miotic response than is obtained from pilocarpine solutions.

Ophthalmic flakes were prepared by dissolving pilocarpine alginate[1] powder (7% w/v) in sterile water for injection, with the aid of mechanical mixing. The solution was delivered into flat-bottom petri vessels and subjected to evaporation under reduced pressure at 30° in a thermostated water bath assembly. When the colloidal solution exhibited a semisolid consistency, the mass was sectioned into circular flakes (0.3-mm. thickness, 3–7-mm. diameter, 3.1–7.8 mg.) by means of various size trephines and dried to the point of solidification. Following additional drying for 24 hr. at room temperature, the ensuing transparent disks were removed and stored in light-resistant containers. Elemental analysis, on an anhydrous basis (loss on drying 2.94%), showed C = 49.65%, H = 6.39%, O = 38.14%, and N = 6.51%, corresponding to an alkaloid content of 48.54%. (Theoretical[2]: C = 50.76, H = 6.47, O = 35.81, and N = 6.96.)

Pilocarpine alginate, 3.34% (w/v), and pilocarpine hydrochloride, 2.00% (w/v), in the presence of methylcellulose 4000 cps., required to adjust the viscosity of the latter to that of the alginate (72 cps., Brookfield, LVT, 25°) solution, were prepared from sterile Sørensen phosphate buffer stock solutions mixed in varying proportions to give a final pH of 6.14 and adjusted for tonicity with sodium chloride.

Miotic studies were conducted using albino, 4–5-kg., male rabbits. The animals were allowed to equilibrate under constant conditions of illumination for 24 hr. prior to commencing treatment with either solid or liquid doses.

Each solution was delivered from a micrometer syringe (0.075 ml.) into the lower cul-de-sac of one eye, and its vehicle was used as the control in the other eye. With the solid dose studies, flakes were deposited into the lower sac with the aid of forceps after being soaked for 30 sec. in isotonic sodium chloride to allow the disk to assume a semiplastic consistency and reduce the degree of initial contact irritation. Alginic acid flakes, similarly prepared, were used as controls. The size of each pupil was measured immediately before the test drug was applied with an Optiker-Ryser pupillary gauge fixed at a distance of 15.2 cm. (6 in.) from the globe.

Pupillary responses (Fig. 1) indicate that, in the liquid state, pilocarpine alginate exhibits essentially comparable miotic activity as pilocarpine hydrochloride following single-dose treatment. No pupillary constriction was noted in both liquid and solid dose control eyes. The results derived from solid pilocarpine alginate deposition show the magnitude of maximum pupil size constriction to be enhanced, with duration of miosis significantly increased over that of both liquid dosage systems. Restoration of normal pupillary diameter for the solid-state dose is observed to occur between 7 and 8 hr. in contrast to about 3.5 hr. for the ophthalmic solutions.

These results indicate that availability of pilocarpine in the cul-de-sac from solid doses may be more uniform as a consequence of diminished diffusion through the gel matrix where the drug is held in reserve, in contrast to liquid dosage forms where the dose is immediately released in the conjunctival fluids. The present observations suggest that the use of solid ophthalmic dosages in the treatment of glaucoma may be more effective, requiring less frequent administration of drug to produce a prolonged physiological activity.

(1) N. Shaftel and A. Halpern, *Amer. J. Med. Sci.*, **236,** 184 (1958).

(2) A. Halpern, N. Shaftel, and G. Schwartz, *Antibiot. Chemother.*, **9,** 97(1959).

(3) A. Halpern, N. Shaftel, and A. J. Montebovi, *Amer. J. Pharm.*, **130,** 190(1958).

(4) S. P. Loucas and H. M. Haddad, Physiology and Pharmacology Symposium: Recent Advances in Drug Dynamics and Drug Delivery Systems in the Eye, Assoc. Res. Vision. Ophthal., Florida, 1971.

S. P. Loucas▲
H. M. Haddad
Department of Ophthalmology
New York Medical College
Flower and Fifth Avenue Hospitals
New York, NY 10029

Received January 13, 1972.
Accepted for publication March 7, 1972.
▲ To whom inquiries should be directed.

[1] Tilden-Yates Laboratories, Inc.

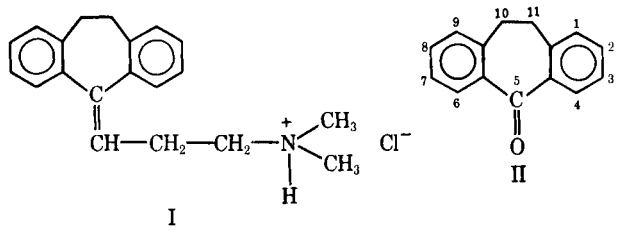
[2] Estimated as a percent composition based on an empirical formula of $(C_6H_8O_6 \cdot H_2O)_n$ and $C_{11}H_{16}N_2O_2$ for alginic acid and pilocarpine, respectively.

Anthraquinone, a New Chemical Oxidation Product of Amitriptyline

Keyphrases □ Amitriptyline oxidation, permanganate—identification of dibenzosuberone and anthraquinone as products □ Dibenzosuberone and anthraquinone—identification as oxidation products of amitriptyline □ Anthraquinone and dibenzosuberone—identification as oxidation products of amitriptyline

Sir:

Wallace and Dahl (1) described a quantitative UV spectrophotometric procedure for the determination of amitriptyline (I) and nortriptyline as well as their prin-



I II

cipal metabolites in biologic specimens. In their procedure, the drugs are extracted into *n*-hexane and oxidized with buffered permanganate to carbonyl derivatives which, in contrast to the original compounds, absorb strongly at 250 nm. In their discussion, the authors suggested the formation of a diconjugated ketone: dibenzosuberone (5*H*-dibenzo-10,11-dihydro-[*a*,*d*]cyclohepten-5-one) (II). This chemical was also isolated by Henwood (2). This author detected the compound as a result of the autoxidation of amitriptyline base.

By submitting the pure drug, as well as urine from humans and rabbits administered the same compound, to the oxidative procedure mentioned, we finally isolated a compound completely different from the described dibenzosuberone. After crystallization from chloroform, it melted at 286° (corrected), while the melting point of dibenzosuberone is 28°. The NMR spectrum measured in deuterochloroform against tetramethylsilane confirmed the presence of aromatic protons (1.7 and 2.2 p.p.m.), while no methylenic proton was detected in the 7-p.p.m. region. The IR spectrum of the compound (KBr pellet) was identical to that of anthraquinone. On the other hand, both the absorption bands at 2925 and 2850 cm.$^{-1}$, corresponding to antisymmetrical and symmetrical vibrations of methylenic CH, respectively, were absent. The centesimal analysis was in agreement with the anthraquinone formula and, in mixture with authentic anthraquinone, no depression of the melting point was observed.

It clearly appears that, by boiling amitriptyline in a permanganate alkaline solution, anthraquinone is almost quantitatively obtained instead of dibenzosuberone, the product of self-decomposition of amitriptyline at room temperature. However, it is possible that dibenzosuberone is formed previously as the first step of this reaction. The confusion between anthraquinone and dibenzosuberone could be explained as follows. If we consider the strong absorption band at 1680 cm.$^{-1}$ alone, it may correspond to a carbonyl function conjugated to two aromatic rings, which is the case for both dibenzosuberone and anthraquinone. However, the latter marks a slight shift due to the fact that only one antisymmetrical stretching vibration, resulting from the coupling of both carbonyls, is visible in IR. With regard to the GLC experiment, the use of a slightly polar phase results in almost identical retention times

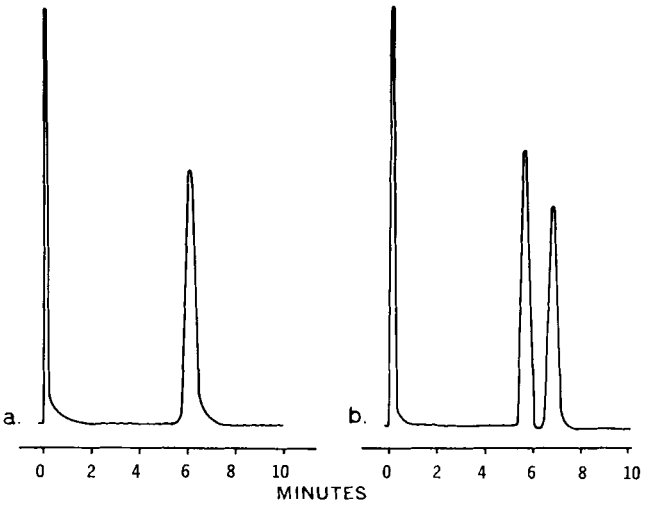


Figure 1—*GLC of a mixture of dibenzosuberone and amitriptyline oxidation product. (a) Column 3% SE-30 on Chromosorb W (60–80 mesh), 2.4-m. by 0.6-cm. (8-ft. by 0.25-in.) glass column; column temperature, 190°; gas flow, 20 ml. nitrogen/min. (b) Column 50% BDS 1.7% + 50% XE-60 2% on Chromosorb G (AW-DCMS) (80–100 mesh), 1.8-m. by 0.6-cm. (6-ft. by 0.25-in.) glass column; column temperature, 225°; gas flow, 40 ml. nitrogen/min.*

for the dibenzosuberone and the anthraquinone; once mixed, they become extremely difficult to separate. However, trapping of the dibenzosuberone at the outlet of the chromatograph and the subsequent recording of an IR spectrum in KBr micropellet clearly show that the dibenzosuberone remains unchanged, in spite of the fact that this substance spontaneously converts into anthraquinone at 200° in the presence of oxygen. The use of a more polar liquid phase as well as an increased temperature allows the separation of both substances (Fig. 1).

On the other hand, the procedure described by Wallace and Dahl (1) still is helpful for identifying amitriptyline and homologs. Following the described procedure, most of the dibenzocycloheptenic compounds, as 10- or 10,11-hydroxy-, mono- or didemethylated amitriptyline, are finally converted into anthraquinone. This last compound is stable in oxidizing media; its separation by steam distillation is quantitative and its determination by GLC presents no difficulty. A recovery of 90% is easily obtained.

(1) J. E. Wallace and E. V. Dahl, *J. Forensic Sci.*, **12**, 484(1967).
(2) C. R. Henwood, *Nature*, **216**, 1039(1967).

R. BOUCHE
Laboratoire de Toxicologie
Ecole de Pharmacie
Université Catholique de Louvain
4, Van Evenstraat
3000-Louvain, Belgium

Received October 4, 1971.
Accepted for publication February 11, 1972.