The purpose of these experiments was to check the hypothesis that a direct interaction between fatty acid molecule and chemical transmitters of noradrenergic systems can account for sleep induction (Rizzoli & Galzigna, 1969). The present experiments do not exclude such an hypothesis.

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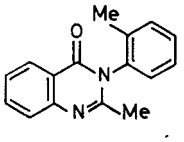
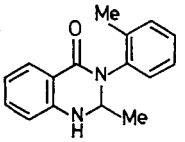
Fluorimetric assay of methaqualone in plasma by reduction to 1,2,3,4-tetrahydro-2-methyl-4-oxo-3-*o*-tolylquinazoline

There is a need for a method of assay of therapeutic plasma levels of the hypnotic drug methaqualone (I). Several indirect methods of estimating the drug necessitate acid or alkaline hydrolysis to diazotizable amines (Maggiorelli & Gangemi, 1964; Nakano, 1964), but these lack specificity. Ultraviolet spectrophotometry offers more attractive quantitative procedures (Akagi, Oketani & Takada, 1964; Lawson & Brown, 1967), but the sensitivity is restricted by interference from biological blanks.

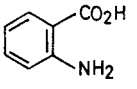
The structural resemblance between the dihydro-derivative of methaqualone (II) and anthranilic acid (III), an efficient fluorophor, suggested that a fluorimetric assay might be developed for methaqualone if a suitable reducing agent could be found. Okumura, Oine & others (1968) reduced some quinazolinone hydrochlorides with sodium borohydride, but the free bases were resistant to this reagent except under conditions which led to ring scission. We have found, however, that lithium borohydride is effective in reducing both free methaqualone and its hydrochloride to the tetrahydroquinazolinone



I



II



III

(II) and that this is stable in the presence of a large excess of reagent. Since (II) does show intense ultraviolet fluorescence, its preparation has been made the basis of a highly sensitive and specific assay for methaqualone in plasma.

Preparation and characterization of 1,2,3,4-*tetrahydro*-2-*methyl*-4-*oxo*-3-o-*tolyl*-*quinazoline* (*II*). An ice-cooled, stirred suspension of methaqualone hydrochloride (500 mg) in tetrahydrofuran (10 ml; freshly redistilled from sodium) was treated dropwise during 5 min with a suspension of lithium borohydride (60 mg; Fluka) in tetrahydrofuran (10 ml.) Completion of the reaction was indicated by dissolution of the hydrochloride, and development of a yellow colour. After stirring (15 min), water (2 ml) was added, and the solution evaporated to dryness. The residue was triturated with water (2 × 10 ml), desiccated, and recrystallized from ethyl acetate to give the tetrahydroquinazolinone (250 mg), as rhombs m.p. 197–8°. The findings in Table 1 confirm the structure assigned to the quinazolinone and show its freedom from methaqualone.

Table 1. *Comparison of some chromatographic and spectral properties of methaqualone and* 1,2,3,4-*tetrahydro*-2-*methyl*-4-*oxo*-3-o-*tolylquinazoline*

Property	Methaqualone	Tetrahydroquinazolinone
Rf, thin-layer chromatography on MN-polyamide; aqueous ethanol	0·72	0·51
Rf, thin-layer chromatography on silica; chloroform–acetone–acetic acid	0·62	0·69
Retention time (min); gas chromatography on SE 30 at 187°	10·5	20
Infrared absorption maxima (μm); ATR spectra on KRS plates—		
C=O	6·0, strong	6·2, strong
C=N	6·3, strong	— absent
N—H	— absent	3·1, medium
Ultraviolet absorption maxima in ethanol— λ_{max}nm (log E)	225 (4·6)	223 (4·5)
	266 (4·0)	—
	295 (3·5)	—
	305 (3·6)	—
	316 (3·4)	—
	—	342 (3·5)
Fluorescence spectra in methanol; activation/emission maxima (nm)	none	345/450

Fluorescence spectra of the tetrahydroquinazolinone were measured with an Aminco-Bowman spectrophotofluorimeter (xenon arc lamp with off-axis ellipsoidal mirror; 1 P 21 photomultiplier). The fluorescence characteristics in aqueous buffers were similar to those in organic solvents, and showed little change between pH 3–10.

At the activation and emission optima, linear log-log working curves for the various slit arrangements are obtained over a wide range of concentrations (0·01 to 100 μg/ml). The limit of detection of the pure compound is about 2 ng in 0·2 ml. The activation optimum at 345 nm, corresponding to the minor absorption peak in the ultraviolet spectrum at this wavelength, is shifted to 365 nm if a xenon/mercury arc is used as the exciting source, because of increased light output by the lamp at this wavelength. Under these conditions, the narrower slit arrangements can be employed for a given instrumental sensitivity, and this facilitates the use of microcuvettes. Efficient activation at the mercury emission line would also provide an advantage when working with filter fluorimeters not equipped with a white light source.

Micro assay of methaqualone in plasma. Heparinized plasma (50 μl) is extracted with Analar ethyl acetate (2 ml; redistilled) on a mechanical shaker for 10 min. After brief centrifugation, an aliquot (1·8 ml) of the supernatant is extracted with 0·05 M sodium hydroxide (50 μl), and separated similarly. The organic phase (1·6 ml) is evaporated to dryness, and the residue treated with a solution of lithium borohydride in anhydrous ether (2 ml of the supernatant from a freshly prepared suspension of nominal concentration 2 mg/ml). After 20 min, a small precipitate is removed by centrifugation and the solution evaporated to dryness. The residue is treated with Analar methanol (1 ml) to hydrolyse the reduction complex and unchanged reagent, and the solution is left for a few minutes until effervescence ceases. An aliquot is then taken for measurement of the fluorescent intensity relative to known standards and to a reagent blank. Suitable concentrations of the tetrahydroquinazolinone itself may be used for day-to-day standardization of the fluorimeter; alternatively, known amounts of methaqualone may be added to normal plasma and carried through the reaction sequence. Recoveries in the range 80 to 90% were readily obtained in this way with 0·1 to 6 μg quantities of methaqualone added to 50 μl samples of normal plasma, in spite of the fact that the drug is strongly protein bound.

By extracting methaqualone from plasma at physiological pH and washing the extract with alkali, a large measure of specificity can be achieved since many common drugs and their metabolites are acidic in character. Thus the barbiturates, salicylate and paracetamol do not interfere with the estimation. Interference might be expected from substances such as glutethimide, meprobamate or methyprylone, but these compounds have not been found to give rise to fluorescent derivatives under the conditions of the assay. Drugs which are themselves strongly fluorescent, and which would remain in the organic phase, such as quinidine or lysergic acid diethylamide might also interfere with the method, but this could be checked by measuring the fluorescence of the extract in a suitable solvent before carrying out the reduction.

This procedure can be used without modification for the assay of plasma methaqualone in poisoned patients, in whom levels almost invariably exceed 5 μg/ml (Matthew, Proudfoot & others, 1968). For the determination of therapeutic levels of the drug, 100 or 150 μl aliquots of plasma are taken, so as to give a final fluorescent intensity of at least twice that of the reagent blank. The blank itself is largely derived from the excess of lithium borohydride which, after methanolysis, shows weak fluorescence (activation/emission maxima, 310/375 nm).

In specimens from 15 patients on therapeutic regimes of Mandrax (Roussel), plasma levels of methaqualone approximately 12 h after ingestion were found to range from 0·9 to 2·2 μg/ml (mean and standard deviation, 1·5 $\pm$ 0·5 μg/ml).

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