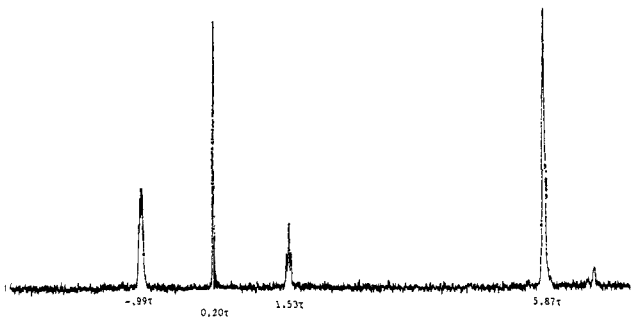


Figure 1.

The ion was found to be stable up to at least 150°. Nmr line broadening was observed over the temperature range 85–112°. (At 42°, the line widths for protons 1, 2, and 3, respectively, were 8.5, 8, and 4 Hz. At 112° the line widths were 19.5, 20, and 8 Hz.) We believe that this broadening is due to a reversible 1,2-hydride shift to B. This mechanism leads to matrix I

Matrix I

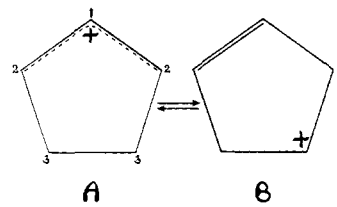
$$\begin{vmatrix} & 1/2 & 0 \\ 1/4 & & 3/8 \\ 0 & 3/16 & \end{vmatrix}$$

of transition probabilities between the spectral lines for a single step of the mechanism. The possibility of a direct 1,4-hydride shift which if suprafacial would be forbidden as a thermal process was considered, and this mechanism would lead to matrix II. Both of these

Matrix II

$$\begin{vmatrix} & 1 & 0 \\ 1/2 & & 1/2 \\ 0 & 1/4 & \end{vmatrix}$$

matrices indicate that there is direct proton exchange between sites 1 and 2 and between sites 3, but no exchange takes place, in a single mechanistic step, between sites 1 and 3. Double resonance experiments at 42° were in agreement with both mechanisms; irradiating



line 1 decreased the integral of line 2 but not line 3. Irradiating line 3 decreased the integral of line 2 but not line 1. These results demonstrate direct exchange between sites 1 and 2 and also 2 and 3, but no direct exchange between 1 and 3. Theoretically calculated curves, computed using matrix I (1,2-hydride shift), agreed satisfactorily with the experimental spectra but using matrix II (that for the 1,4-hydride shift) agreement could not be obtained.

Fitting the rates obtained at different temperatures for the 1,2-hydride shift to the Arrhenius equation gave $E_a = 18.0 \pm 0.9$ kcal/mol and $\log A = 12.2 \pm 0.6$ where errors reported are standard deviations.

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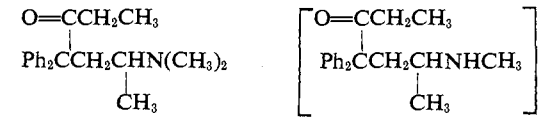
The Identification of Three New Metabolites of Methadone in Man and in the Rat

Sir:

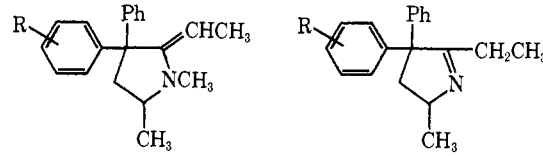
The increasingly wide use of methadone (**1**) in the maintenance therapy of heroin addicts has led to a renewed interest in the metabolic fate of methadone in man and in laboratory animals. In addition to its inherent scientific interest such information is required for a full understanding of the pharmacodynamics of methadone in maintenance subjects.

The initial step in the biotransformation of methadone, in both the rat and in man, is known to occur *via* N-demethylation.[1,2] The de-*N*-methylmethadone (**2**)



methadone (**1**) de-*N*-methylmethadone (**2**) (hypothetical)

resulting from the N-demethylation of methadone has, however, never been directly isolated. Chemical studies[2] have shown that, once formed, **2** spontaneously cyclizes to 1,5-dimethyl-3,3-diphenyl-2-ethylidenepyrrolidine (**3a**). Indeed compound **3a** and its N-de-



3a, R = H
3b, R = OH
3c, R = OCH_3
3d, R = OAc

4a, R = H
4b, R = OH
4c, R = OCH_3
4d, R = OAc

methyl analog, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (**4a**), have been identified as metabolites of methadone in both man and rat.[2,3]

In the course of our studies on the fate of methadone in subjects receiving a daily 80-mg maintenance dose it became clear that urine samples contained metabolites in addition to those already reported (see above). These new metabolites were relatively polar and remained in urine following the removal of **1**, **3a**, and **4a** by extraction with butyl chloride.[4] They were recovered and identified as follows.

Aliquots (100 ml) of extracted urine were adjusted to pH 7 and placed on an Amberlite XAD-2 (Rohm and Haas) column (2 cm × 36 cm) and washed with 200 ml of water. The metabolites were eluted from the column

(1) J. Axelrod, *J. Pharmacol. Exp. Ther.*, **117**, 322 (1956).
(2) A. Pohland, H. R. Sullivan, and H. M. Lee, Abstracts, 136th National Meeting of the American Chemical Society, Sept 1959, Atlantic City, N. J., p 15-O.
(3) A. Pohland, H. E. Boaz, and H. R. Sullivan, *J. Med. Chem.*, **14**, 194 (1971).
(4) H. R. Sullivan and D. A. Blake, *Res. Commun. Chem. Pathol. Pharmacol.*, in press.

with 100 ml of methanol and were recovered by evaporation of the solvent. These metabolites were presumed to be conjugates of hydroxylated compounds since they were readily hydrolyzable to less polar entities by incubation with a mixture of β-glucuronidase and aryl sulfatase (Glusulase, Endo products) at pH 5.5. That they were hydroxylated materials was confirmed as follows.

The hydrolyzed metabolites were recovered by extraction into CH_2Cl_2 and were examined by combined gas chromatography–mass spectrometry (Model LKB 9000) using an 8-ft column of 1% W-98 silicone gum rubber on Gas Chromosorb Q at 165°. Under these conditions GC peaks were seen with retention times of 9 and 11 min. The metabolite with the longer retention time had a molecular ion of 293 and a fragmentation pattern consistent with structure **3b**, *i.e.*, hydroxylated **3a**.

Conversion of **3b** to **3c** ($M^+ = 307$) by treatment with diazomethane confirmed the presence of a phenolic hydroxyl. Reaction of **3b** with acetic anhydride yielded **3d** ($M^+ = 335$). The fragmentation patterns of **3b**, **3c**, and **3d** were all consistent with the assigned structure.

Similarly the metabolite with the shorter retention time was found to have the structure **4b**. Reaction with diazomethane converted **4b** to **4c** while **4d** was formed by reaction with acetic anhydride. The mass fragmentation patterns of **4b** ($M^+ = 279$), **4c** ($M^+ = 293$), and **4d** ($M^+ = 321$) were all consistent with the structures assigned.

Enzymatic hydroxylation of an aromatic ring is a well-known pathway in the metabolism of exogenous substances in mammals (*cf.* Daly[5] for a recent review of aromatic hydroxylation and the role of arene oxide intermediates). Para hydroxylation is usually observed in the case of monosubstituted benzene rings. For example, para hydroxylation is the major pathway in the metabolism of diphenylhydantoin,[6] although meta hydroxylation has been observed as a minor pathway.[7] It is likely that metabolites **3b** and **4b** are *p*-hydroxy compounds, but this must be established by synthesis or additional physical studies. A significant point, however, is that although aromatic hydroxylation is an important pathway it has not as yet been reported as a route of metabolism of synthetic opiates.[8]

In order to obtain further information about **3b** and **4b**, the fate of radiocarbon-labeled methadone was studied in rats. Both **3b** and **4b** were found to be excreted as radioactive conjugates (either glucuronides or sulfates) in the bile of rats receiving methadone-*2*-^{14}C. Further confirmation of the nature of **3b** and **4b** came from the observation that the administration of **3a** to rats resulted in the excretion of conjugates of **3b** and **4b** into bile. When **4a** was administered only conjugated **4b** was detected.

In addition to the new metabolites, **3b** and **4b**, a further metabolite was found to be directly extractable from acidified human or rat urine. The chromatographic behavior and mass fragmentation pattern of this metabolite were identical with those of a known sample of 4-dimethylamino-2,2-diphenylpentanoic acid (**5**). This acid would be expected to arise *via* the oxidative removal of C-1 and -2 from methadone. The observation that 1–5% of a dose of methadone-*2*-^{14}C in the rat is expired as radiocarbon dioxide is consistent with this suggestion.

The formation of the carboxylic acid metabolite **5** has some precedent in the metabolic conversion of acylbenzenes to benzoic acid.[9] However, the usual route of metabolism of ketones is *via* enzymatic reduction,[10] a reaction which may be suppressed in methadone metabolism because of the highly hindered nature of this ketone.

The quantitative importance of these new metabolites has not as yet been completely established. However, their relative abundance in the urine of a typical methadone maintenance subject appears to be: **3a**, **4b** > **1** > **3b** > **4a**, **5**. In addition to further studies on metabolite quantitation we are also directing our attention to a resolution of the isomer problem represented by structures **3b** and **4b**.

(5) J. Daly, *Handb. Exp. Pharmacol.*, **24**, (2), 285 (1971).
(6) T. C. Butler, *J. Pharmacol.*, **119**, 1 (1957).
(7) A. J. Atkinson, J. MacGee, J. Strong, D. Garteiz, and T. E. Gaffney, *Biochem. Pharmacol.*, **19**, 2483 (1970).
(8) E. L. Way and T. K. Adler, "The Biological Disposition of Morphine and its Surrogates," World Health Organization, Geneva, Switzerland, 1962.
(9) R. T. Williams, "Detoxication Mechanisms," 2nd ed, Wiley, New York, N. Y., 1959, pp 335–338.
(10) R. E. McMahon, *Handb. Exp. Pharmacol.*, **24** (2), 500 (1971).

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Characterization of an Acid Hydrolysis Product of Starfish Toxins as a 5α-Pregnane Derivative

Sir:

Starfish contain toxins which are cytotoxic, hemolytic, antiviral, and repellent to other marine animals.[1] The author has recently reported the purification of the toxins from three species of starfish.[2]

A toxin, ASP-II (**1**), from crown of thorns, *Acanthaster planci*,[3] is of glycosidic nature, and has a sulfate group and a molecular weight of about 1500 (*Anal.* Found: S, 2.08%). Upon hydrolysis with 2 *N* H_2SO_4, **1** gave sugars (quinovose and fucose in about a 2:1 ratio) and the aglycone fraction as a mixture of closely resembled compounds, from which, after repetitions of chromatography, a pure crystalline compound **2** was obtained. **2** constitutes about one-third of the aglycone fraction, and it was also isolated from the hydrolysate of toxins of *Asterias forbesi*, an Atlantic species.[3]

The compound **2**, long needles from methanol–water, mp 193–196°, $[\alpha]^{23}D$ +98.4° (methanol), was given the molecular formula $C_{21}H_{32}O_3$ on the basis of the mass spectrum and elemental analysis (M^+, *m/e* 332. *Anal.* Found: C, 75.62; H, 9.50%). The ir spectrum showed the presence of hydroxyl groups (3400 cm^{-1}) and a carbonyl absorption (1700 cm^{-1}). The absence of a conjugated system in **2** is evident from the lack of strong absorptions in the uv spectrum. In the mass spectrum, the base peak is acetylium ion, *m/e*

(1) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," Vol. 1, U. S. Government Printing Office, Washington, D. C., 1965, p 537, and also see the references cited in ref 2.
(2) Y. Shimizu, *Experientia*, **27**, 1188 (1971).
(3) *Acanthaster planci* was collected in Hawaii. *Asterias forbesi* was collected locally in May 1971.