A New Benzylisoquinoline Alkaloid: *N*-Methylpalaudinium Chloride

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Abstract □ A new benzylisoquinoline alkaloid, *N*-methylpalaudinium chloride (I), was isolated from *Thalictrum polygamum* Muhl. (Ranunculaceae).

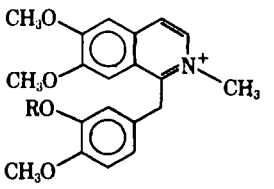
Keyphrases □ *N*-Methylpalaudinium chloride—isolation and identification from *Thalictrum polygamum* □ *Thalictrum polygamum*—isolation and identification of *N*-methylpalaudinium □ Benzylisoquinoline alkaloids—isolation and identification of *N*-methylpalaudinium from *Thalictrum polygamum*

During an investigation of the quaternary alkaloids of *Thalictrum polygamum* Muhl. (Ranunculaceae)[1], a new aromatic and quaternary benzylisoquinoline alkaloid, *N*-methylpalaudinium chloride (I), $C_{20}H_{22}ClNO_4$, m.p. 232–233°, was isolated.

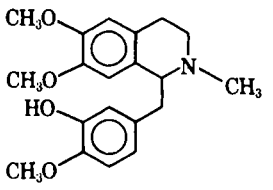
The UV spectrum of I exhibited $\lambda_{max.}^{MeOH}$ 228, 256, 286, 315, 330 sh, and 350 sh nm. (log ϵ 4.40, 4.75, 3.86, 4.02, 3.89, and 3.75), a pattern very similar to that of papaverine methiodide (II) which shows $\lambda_{max.}^{MeOH}$ 226, 285, 318, 240 sh, and 352 sh nm. (log ϵ 4.52, 4.66, 3.79, 4.02, 3.97, and 3.75).

The NMR spectrum of I was also close to that of II, the main difference being the presence of only three aromatic methoxy signals in the former as opposed to four such signals in the latter salt. The existence of a phenolic function in I was indicated by a one proton signal at δ 9.25 exchangeable with deuterium oxide and by the formation of the monoacetate derivative III.

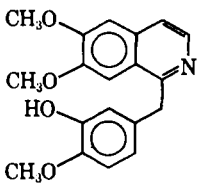
Sodium borohydride reduction of I afforded the known base (±)-laudanine (IV) (1), thus determining the position of the phenolic function in ring C. Final confirmation of the structural assignment was obtained when it was found that treatment of a sample of the alkaloid palaudine (V) (2) with methyl iodide followed by ion exchange afforded palaudine methochloride, identical with the natural product.



I: R = H
II: R = CH_3
III: R = Ac



IV



V

Benzylisoquinoline alkaloids with an aromatic ring B as in I, II, or V have so far been found in members of the Papaveraceae, Ranunculaceae, and Menispermaceae families. Known alkaloids, which were also found in the present study of *T. polygamum*, were the aporphine (+)-magnoflorine and the protoberberine salts berberine and thalifendine.

EXPERIMENTAL[2]

Isolation of *N*-Methylpalaudinium Chloride (I)—The crude aqueous extract from 5 kg. of finely ground *T. polygamum* after removal of fats and the tertiary alkaloids (3) was made basic with ammonium hydroxide and was treated with a warm saturated solution of ammonium reineckate to precipitate the quaternary amines. The precipitate was collected, dissolved in acetone, and passed through an ion-exchange column[3] using acetone–methanol (1:1). The total eluate was evaporated and chromatographed on neutral alumina using acetone–methanol mixtures. The fraction eluted with 75% acetone–25% ethanol consisted of one major compound by TLC analysis (blue color with iodoplatinate spray reagent) (4).

Evaporation of these fractions gave a residue which crystallized from methanol–acetone. Recrystallization from methanol–acetone gave colorless needles (0.2 g.) of the quaternary chloride (I), m.p. 232–233°; mass spectrum: *m/e* 340 (M^+), 338 (100), 323 (100), 309 (30), 295 (16), 279 (10), 266 (7), 250 (7), 238 (8), 222 (7), 204 (25), 169.5 (11), 151 (40), and 137 (25); NMR (dimethyl sulfoxide): δ3.75 s (3H, C-4, OCH_3), 4.03 s (3H, C-7, OCH_3), 4.11 s (3H, C-6, OCH_3), 4.38 s (3H, $^+N—CH_3$), 6.55 d (1H, J=8 Hz., C-6′*H*), 6.60 broad s (1H, C-2′*H*), 6.88 d (1H, J=8 Hz., C-5′*H*), 7.80 s (1H, C-8*H*), 7.90 s (1H, C-5*H*), 8.32 and 8.65 *AB* quartet (2H, J=7 Hz., C-3*H*, C-4*H*), and 9.25 s (1H, phenolic O*H*).

Anal.—(High-resolution mass spectrum): Calc. for $C_{20}H_{22}NO_4$: 340.1547. Found: 340.1526 (error = 6.2 p.p.m.).

Preparation of *O*-Acetyl-*N*-methylpalaudinium Chloride (III)—Acetic anhydride (0.5 ml.), pyridine (0.5 ml.), and I (25 mg.) were combined and allowed to stand for 16 hr. at 25°. Methanol was added and the mixture was evaporated to dryness. The residue was dissolved in a methanol–acetone mixture and stored at 0°, whereupon crystals of II separated. Recrystallization from cold methanol–acetone gave 21 mg. of III as light-yellow needles, m.p. 120–122°; $\nu_{max.}^{KBr}$ 1770 cm.$^{-1}$ (acetate carbonyl); $\lambda_{max.}^{EtOH}$ same as for I. Mass spectrum: *m/e* 383 (M^+) (7), 380 (44), 366 (33), 352 (12), 337 (12), 323 (100), 310 (50), 296 (12), 239 (10), 204 (11), 183 (15), 179 (25), and 137 (41); NMR ($CDCl_3$): δ2.22 s (3H, acetate CH_3), 3.76 s (3H, C-7, OCH_3), 4.00 s (3H, C-4′, OCH_3), 4.12 s (3H, C-6, OCH_3), 4.58 s (3H, $^+N—CH_3$), 6.82–6.88 m (3H, C-2′, C-3′, C-5′*H*), 7.50 s (1H, C-8*H*), 7.68 s (1H, C-5*H*), and 8.25 and 8.65 *AB* quartet (2H, J=7 Hz., C-3, C-4*H*).

Anal.—(High-resolution mass spectrum): Calc. for $C_{22}H_{24}NO_5$: 382.1648. Found: 382.1624 (error = 6.3 p.p.m.).

[1] The plant was collected in central Pennsylvania in July 1969. A voucher specimen is deposited with the herbarium in the Department of Botany, Pennsylvania State University, University Park, Pa. The plant samples were identified by Prof. H. A. Wahl, Department of Botany, Pennsylvania State University.

[2] Melting points were obtained on a melting-point block and are uncorrected. NMR spectra were recorded on a Varian A-60A and HA-100. The mass spectra were measured on an Atlas AEI-MS9. TLC was on Brinkmann silica gel and aluminum oxide plates; visualization was by spraying with an iodoplatinate reagent.

[3] Amberlite CG-400, chloride form.

Preparation of (±)-Laudanine (IV) from I—Sodium borohydride (0.1 g.) was added in portions to a solution of I (0.1 g.) in ethanol and the mixture was stirred at room temperature for 3 hr. Methanol was added and after 30 min. the reaction mixture was evaporated to dryness. The residue was dissolved in water and extracted with ether. The ether extracts were dried and evaporated to yield a residue which crystallized from methanol as white prisms of IV, m.p. 166–168°; $\lambda_{max.}^{EtOH}$ 230 and 283 nm. (log ε 4.08 and 3.78); positive $FeCl_3$ test, violet Burger test; mass spectrum: *m/e* 343 (1), 342 (5), 327 (2), 206 (100), 204 (8), 192 (40), 190 (80), 174 (8), 162 (36), and 137 (19); NMR ($CDCl_3$): δ2.52 s (3H, N—CH_3), 3.58 s (3H, C-7, OCH_3), 3.85 s (6H, C-6, C-4′, OCH_3), 6.08 s (1H, C-8*H*), 6.58 s (1H, C-5*H*), 6.52 and 6.73 *AB* quartet (2H, *J* = 8 Hz., C-2′, C-3′*H*), and 6.75 broad s (1H, C-5′*H*); (±)-laudanine lit. (1) m.p. 166–167°, Burger test: violet color (1).

Interconversion of Palaudine and I—A solution of V (3 mg.) and methyl iodide (0.1 ml.) in ethanol (2 ml.) was allowed to stand for 18 hr. It was evaporated and passed through an ion-exchange column (Cl^- form) using ethanol–acetone. The residue was crystallized from ethanol as needles (4 mg.), m.p. 231–233°. Palaudine methochloride and I were indistinguishable in terms of the R_f values and UV spectra, and no mixed melting-point depression was observed.

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(4) "Thin Layer Chromatography," 2nd ed., E. Stahl, Ed., Springer-Verlag, New York, N. Y., 1969, p. 883.

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Metabolism of Aniline and Hexobarbital by Liver Homogenates of Spironolactone-Pretreated Male Rats

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Abstract □ Spironolactone pretreatment increases aniline hydroxylation and decreases hexobarbital metabolism, with respect to the saline-treated controls, by liver homogenates of male rats. The effect of spironolactone pretreatment on the metabolism of a substrate is related to the sex dependence or independence of that substrate's metabolism. No spironolactone-induced changes in microsomal protein, cytochrome P-450 content, or carbon monoxide-induced P-450 difference spectra were observed.

Keyphrases □ Aniline—metabolism by liver homogenate of spironolactone-pretreated male rats □ Hexobarbital—metabolism by liver homogenate of spironolactone-pretreated rats □ Spironolactone pretreatment—effects on aniline and hexobarbital metabolism, liver homogenates, male rats

The effect of spironolactone on the NADPH-dependent microsomal hydroxylation pathway of drug metabolism has been the subject of several recent investigations. Solymoss *et al.* (1) and, more recently, Stripp *et al.* (2) showed that spironolactone enhances hexobarbital degradation in female rats. However, the latter investigators also showed that spironolactone retards hexobarbital degradation in male rats (2). These results suggest that the effect of spironolactone on drug metabolism is a function of sex.

The metabolism of hexobarbital in the rat is sex dependent (3), but the metabolism of aniline in this animal is independent of sex (4). Therefore, the authors investigated the *in vitro* metabolism of these substrates in rat liver homogenates from male animals pretreated with spironolactone, hoping to obtain insight into the nature of this drug's effect upon the metabolism of other drugs.

MATERIALS AND METHODS

Animals—Male weanling rats[1], maintained on Purina Laboratory Chow and tap water *ad libitum*, were used experimentally upon attaining a weight of 125–135 g. One group of seven experimental animals was injected intraperitoneally with 100 mg./kg. spironolactone[2] in saline twice daily for 4 days, according to the method of Stripp *et al.* (2). Another group of seven control animals received isotonic saline injections. Animals were fasted overnight prior to use and were sacrificed 16 hr. after the final injection.

Liver Homogenates—Animals were sacrificed, and 10% liver homogenates were prepared in a 0.25 *M* sucrose solution containing 0.05 *M* tromethamine chloride (pH 7.4), 0.005 *M* $MgCl_2$, and 0.010 *M* NaCl as previously described (5).

Aniline Incubations—Aniline (200 μmoles in 0.10 ml. 95% ethanol) was added to a 125-ml. conical flask containing 1.25 ml. of the 0.25 *M* sucrose solution described, which also contained 4 μmoles of $NADP^+$, 14 mg. of glucose 6-phosphate, 0.015 *M* nicotinamide, and 0.1% bovine serum albumin. Ten milliliters of 10% liver homogenate was then added, followed by 5 units (0.5 ml.) of glucose 6-phosphate dehydrogenase in the 0.25 *M* sucrose solution. This reaction mixture was then incubated and assayed for the *p*-aminophenol formed after 20 min. by the method of Kato and Gillette (4). Results were expressed in nmoles of *p*-aminophenol that formed per minute per milligram microsomal protein ± *SE* from six determinations.

Hexobarbital Metabolism—Hexobarbital (1 μmole in 0.10 ml. 95% ethanol) was added to 125-ml. conical flasks containing the incubation medium and the NADPH-generating system already described. The homogenate and the enzyme were added as described for the aniline experiments, and the reaction mixtures were incubated and assayed for hexobarbital disappearance after 1 hr. by the method of Cooper and Brodie (6). Results were expressed in nmoles of hexobarbital that disappeared per minute per milligram microsomal protein.

[1] Obtained from Sasco, Inc., Omaha, Neb.
[2] Obtained from Calbiochem.