TABLE II—ACCURACY DATA FOR THE GLC METHOD

| No. | Mg. Added | Mg. Found[a] | % Recovery ($\bar{x}$)[b] |
|---|---|---|---|
| 1 | 2.0 | 1.95 | 97.5 |
| 2 | 2.0 | 1.96 | 98.0 |
| 3 | 2.0 | 2.02 | 101.0 |
| 4 | 2.0 | 2.04 | 102.0 |
| 5 | 2.0 | 2.01 | 100.5 |

[a] $\sigma = \pm 1.97\%$. [b] $\bar{x} = 99.8$.

TABLE III—REPRODUCIBILITY DATA FOR THE ASSAY METHOD ON FRESH TABLETS[a]

| No. | Mg. Found ($x$) | $(\bar{x} - x)^2$ |
|---|---|---|
| 1 | 2.04 | 0.0009 |
| 2 | 1.96 | 0.0025 |
| 3 | 1.96 | 0.0025 |
| 4 | 2.08 | 0.0049 |
| 5 | 2.01 | 0.0000 |

$\sigma = \pm 0.052$

[a] Each tablet contains theoretical amount of 2 mg. of anagestone acetate.

is a straight line (standard curve). The accuracy of the GLC method was obtained from the data in Table II. The known and found values along with the sigma value (1.97) show that the analysis can be performed satisfactorily. The precision ($\sigma = 0.052$) studies were carried out on fresh tablets and indicated (Table III) the method was sensitive and can be adopted for routine analysis.

Several batches of tablets stored at various temperature stations for different lengths of time were assayed. The results, recorded in Table IV, demonstrate that over a period of 18 months, anagestone acetate does not undergo any alteration.

TABLE IV—ASSAY DATA ON COMBINATION TABLETS[a] FOR ANAGESTONE ACETATE

| No. | Storage Conditions[b] | Mg. Anagestone Acetate per Tablet | % Recovery |
|---|---|---|---|
| 1 | Fresh | 1.96 | 98.0 |
| 2 | RT, 3 weeks | 2.03 | 101.5 |
| 3 | RT, 1 month | 1.98 | 99.0 |
| 4 | RT, 2 months | 2.05 | 102.5 |
| 5 | RT, 15 months | 2.00 | 100.0 |
| 6 | RT, 16 months | 1.97 | 98.5 |
| 7 | 47°, 15 months | 2.02 | 101.0 |
| 8 | 37°, 18 months | 2.01 | 100.5 |
| 9 | 50°, 3 months | 2.04 | 102.0 |

[a] Each tablet contains theoretically 2.0 mg. of anagestone acetate. [b] RT refers to room temperature.


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*Keyphrases*

17-α-ethynylestradiol 3-methylether—anagestone acetate tablets
Anagestone acetate—analysis
GLC—analysis


# The Analgesic, Hypothermic, and Depressant Activities of Some *N*-Substituted α-5,9-Dimethyl-6,7-benzomorphans


By M. MAY, L. CZONCHA, D. R. GARRISON, and D. J. TRIGGLE



**A series of *N*-substituted α-5,9-dimethyl-6,7-benzomorphans bearing *N*-2-hydroxyalkyl and *N*-2-bromoalkyl substituents have been synthesized and their analgesic, hypothermic, and depressant activities determined. The analgesic activities were not outstanding but the *N*-2-bromoethyl- and the *N*-2-bromopropyl derivatives produced prolonged hypothermia and depression. Possible mechanisms of these prolonged actions are discussed.**


It is generally assumed that narcotic analgesics produce their pharmacological effects by interaction at a specific analgesic receptor (or receptors). Extensive work by Beckett and co-workers (1, 2) has resulted in the publication of a tentative description of the analgesic receptor. This description has recently been critically discussed by Portoghese (3) who points out that the analgesic receptor may be more flexible in its binding capability than Beckett originally assumed. Nevertheless, both Beckett and Portoghese assume that one of the principal binding sites at the analgesic receptor is an anionic grouping which is capable of binding, *via* an ion–ion interaction, a protonated amino group of the analgesic molecule.

An anionic binding site (a carboxylate or phosphate anion) is also assumed to be an important fea-


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ture of the structure and function of the adrenergic and cholinergic receptors (4–6), and since it has proved possible to design antagonists which act at these receptors by forming covalent bonds to these anionic sites (4, 7, 8), it appeared of interest to extend this approach to the design of compounds that might interact irreversibly at the narcotic analgesic receptors. This approach, if successful, would yield information valuable in attempted identification of the receptor site(s) and, additionally, might furnish compounds of considerable therapeutic importance.

In this initial approach, the compounds selected for study were *N*-(2-bromoethyl) derivatives (I*b*–III*b*) of α-5,9-dimethyl-6,7-benzomorphan,

I(*a*) X = OH, R = H, (*b*) X = Br, R = H
II(*a*) X = OH, R = $CH_3$, (*b*) X = Br, R = $CH_3$
III(*a*) X = OH, R = Ph, (*b*) X = Br, R = Ph

together with their corresponding nonalkylating parent compounds (I*a*–III*a*). *N*-Methyl-α-5,9-dimethyl-6,7-benzomorphan was employed as an appropriate "standard" analgesic compound (7).

## EXPERIMENTAL

**Chemistry**—Melting points were recorded on a Thomas-Kofler hot stage and are corrected. Analyses are by Dr. A. E. Bernhardt, Mulheim, West Germany.

***N* - Methyl - α - 5,9 - dimethyl - 6,7 - benzomorphan**—This was prepared by the method of May and Fry (9). The hydrochloride salt had m.p. 204° [lit. [9] m.p. 203–204°]. The hydrobromide salt was recrystallized from acetone and had m.p. 188–190°.

*Anal.*—Calcd. for $C_{15}H_{22}BrN$: C, 60.83; H, 7.48; Br, 26.97. Found: C, 60.91; H, 7.22; Br, 27.11.

***N* - (2 - Hydroxyethyl) - α - 5,9 - dimethyl - 6,7-benzomorphan Hydrobromide (Ia)**—To α-5,9-dimethyl-6,7-benzomorphan (10) (4.1 Gm., 0.02 mole) in methanol (20 ml.) was added ethylene oxide (1.3 Gm., 0.03 mole) and the solution was heated to 100° in a steel bomb for 6 hr. On cooling the solution was saturated with hydrogen bromide gas, evaporated under reduced pressure, and the residue dissolved in dry acetone. On cooling crystals of I*a* (74%) were deposited with m.p. 210–212°.

*Anal.*—Calcd. for $C_{16}H_{22}BrNO$: C, 58.9; H, 7.42; Br, 24.49; N, 4.29. Found: C, 59.21; H, 7.17; Br, 24.41; N, 4.51.

***N* - (2 - Bromoethyl) - α - 5,9 - dimethyl - 6,7-benzomorphan Hydrobromide (Ib)**—I*a* (3.3 Gm., 0.01 mole) suspended and stirred in chloroform (20 ml.) at 0° was treated dropwise with a solution of phosphorus tribromide (3.0 Gm., 0.011 mole) in chloroform (10 ml.). The mixture was then refluxed for 4 hr., evaporated under reduced pressure, and the orange residue extracted with boiling acetone (3 × 15 ml.) and filtered through diatomaceous earth. On cooling the filtrate yielded crystals of I*b*, (82%) which were recrystallized (acetone) to a constant m.p. of 195°.

*Anal.*—Calcd. for $C_{16}H_{23}Br_2N$: C, 49.38; H, 5.96; Br, 41.1. Found: C, 49.61; H, 5.71; Br, 40.83.

***N* - (2 - Hydroxypropyl) - α - 5,9 - dimethyl - 6,7-benzomorphan Hydrobromide (IIa)**—This was prepared from α-5,9-dimethyl-6,7-benzomorphan and propylene oxide using the procedure described for I*a*. The product had m.p. 208–215° (82%).

*Anal.*—Calcd. for $C_{17}H_{26}BrNO$: C, 59.99; H, 7.7; Br, 23.48; N, 4.11. Found: C, 60.15; H, 7.31; Br, 23.52; N, 3.98.

***N* - (2 - Hydroxy - 2 - phenylethyl) - α - 5,9-dimethyl-6,7-benzomorphan Hydrobromide (IIIa)**—This was prepared similarly from α-5,9-dimethyl-6,7-benzomorphan and 1,2-epoxyethylbenzene. The product had m.p. 225–235° (92%).

*Anal.*—Calcd. for $C_{22}H_{28}BrNO$: C, 65.63; H, 7.02; Br, 19.86. Found: C, 65.82; H, 6.77; Br, 19.76.

***N* - (2 - Bromopropyl) - α - 5,9-dimethyl - 6,7-benzomorphan Hydrobromide (IIb)**—This was prepared from II*a* and phosphorus tribromide using the procedure described under I*b*. The product had m.p. 180–190° (78%).

*Anal.*—Calcd. for $C_{17}H_{25}Br_2N$; C, 50.61; H, 6.25; Br, 39.64; N, 3.47. Found: C, 50.79; H, 6.51; Br, 39.34; N, 3.50.

***N* - (2 - Bromo - 2 - phenylethyl) - α - 5,9 - dimethyl-6,7-benzomorphan Hydrobromide (IIIb)**—This was prepared similarly from III*a* and phosphorus tribromide. The product had m.p. 168–175° (89%).

*Anal.*—Calcd. for $C_{22}H_{27}Br_2N$: C, 56.76; H, 5.85; Br, 34.34; N, 3.01. Found: C, 56.41; H, 6.13; Br, 34.33; N, 3.21.

## PHARMACOLOGY

Analgesic activity (Table I) was determined using the Eddy-Leimbach modification (11) of the hot-plate method devised by Woolfe and Macdonald (12). Compounds were administered subcutaneously in 0.1 ml. of water. Swiss albino male mice (weight 20–30 Gm.) were employed, 10 animals each dose, and an increase of 10 sec. or more in reaction time from the pretreatment time was taken as an analgesic response (see under *Discussion*).

Spontaneous motor activity (Table II) was mea-

TABLE I—ANALGESIC ACTIVITIES OF *N*-SUBSTITUTED α-5,9-DIMETHYL-6,7-BENZOMORPHANS

| Compd. | $ED_{50}$[a] | Time of Onset, min. | Duration, min. |
|---|---|---|---|
| Standard[b] | 30 | 11 | 28 |
| I*a* | 100[c] | 45 | 30 |
| I*b* | 28 | 80 | 180 |
| II*a* | 48 | 16 | 20 |
| II*b* | 30 | 140 | 180 |
| III*a* | 400[d] | 30 | 20 |
| III*b* | inactive at 400 | | |

[a] Dose in mg./Kg. which produced an apparent analgesic response in 50% of the animals. [b] *N*-Methyl-α-5,9-dimethyl-6,7-benzomorphan. Murphy, Ager, and May (13) report the $ED_{50}$ (as hydrochloride) as 27.3 mg./Kg. [c] Only 40% of the animals affected. [d] Only 30% of the animals affected.

TABLE II—DEPRESSANT ACTIVITIES OF *N*-SUBSTITUTED α-5,9-DIMETHYL-6,7-BENZOMORPHANS

| Compd. | Dose,[a] mg./Kg. | Locomotor Activity,[b] —Time After Injection, %— 15–30 min. | 30–60 min. | 1,455–1,470 min. |
|---|---|---|---|---|
| Water | (control) | 100 | 100 | 100 |
| I*b* | 30 | 87 | 16 | 100 |
| II*b* | 30 | 100 | 17 | 24 |
| Standard | 30 | 189 | 251 | 17 |
| St. + I*b*[c] | 30 + 30 | 337 | 12 | ... |
| St. + II*b*[c] | 30 + 30 | 92 | 20 | ... |

[a] Single subcutaneous injection on first day (time zero). [b] Locomotor activity expressed as % of control activity. [c] In the pretreatment studies standard (St.) was administered 30 min. before I*b* or II*b*.

TABLE III—EFFECTS OF *N*-SUBSTITUTED α-5,9-DIMETHYL-6,7-BENZOMORPHANS ON MICE RECTAL TEMPERATURES

| Compd. | Dose,[a] mg./Kg. | Rectal Temperature, Time After Injection, min. 0 | 30 | 60 | 90 | 120 | 180 | 240 | 24 hr |
|---|---|---|---|---|---|---|---|---|---|
| Dist. water | ... | 37.3 | 37.3 | 37.2 | 37.3 | 37.0 | ... | ... | ... |
| Standard | 70 | 38.4 | 36.5 | 37.4 | 38.3 | 38.5 | ... | ... | ... |
| *Ia* | 100 | 37.3 | 37.3 | 37.1 | 37.1 | 37.1 | ... | ... | ... |
| *Ib* | 40 | 37.1 | 35.6 | 34.6[b] | 33.9 | 33.5 | 33.9 | ... | 37.1 |
| *IIa* | 80 | 37.4 | 36.2 | 36.2 | 36.3 | 37.0 | ... | ... | ... |
| *IIb* | 50 | 37.5 | 34.6 | 33.2[b] | 33.0 | 32.3 | 31.7[b] | 32.9 | 35.7 |
| St. + *1b*[c] | 70 + 40 | 38.1 | 36.2 | 34.5 | 31.9[b] | 30.3 | ... | ... | ... |
| St. + *IIb*[c] | 70 + 50 | 38.2 | 35.8 | 33.7 | 31.2[b] | 29.1 | ... | ... | ... |

[a] 5 animals per dose level. [b] *P* value for significance of difference from standard <0.01. [c] Compound administered 0.5 hr. after standard.

sured using a Lehigh Valley Electronics activity cage (model 1497). The compounds were administered subcutaneously to male mice which were placed immediately in the cage and the locomotor activity determined from the fifteenth to the thirtieth and from the thirtieth to the sixtieth min. Locomotor activity was again determined 24 hr. later. Control animals were treated similarly but received only an equivalent amount of injected water. Locomotor activity of treated mice is expressed as a percentage of the activity of control mice.

Rectal temperatures (Table III) were taken with an Electric Universal thermometer using male mice. Control animals received an equivalent injection of water only. A statistical analysis revealed the significance of the difference in temperature between treated and control mice. In some experiments the effects of prior administration of *N*-methyl-α-5,9-dimethyl-6,7-benzomorphan on the hypothermic and depressant activities of *Ib* and *IIb* were determined. These results are summarized in the bottom columns of Tables II and III.

## DISCUSSION

The analgesic activities of compounds I–III listed in Table I are not outstanding, but it is relevant to note that the differences in each pair of compounds (*a* and *b*) are such as to preclude the possibility that hydrolysis of the 2-bromoethylamine to the 2-hydroxyethylamine is a prerequisite for analgesic activity. However, it is not entirely clear to what extent the effects of *Ib* and *IIb* listed in Table I are due to analgesic or to depressant actions since mice receiving these compounds were found to be profoundly depressed. *Ib* and *IIb* were shown to produce a severe reduction in the locomotor activity of mice (Table II) which, in the case of *IIb*, was still pronounced 24 hr. after administration. None of the other compounds listed in Table I produced depression, indeed, *N*-methyl-α-5,9-dimethyl-6,7-benzomorphan (standard) produced an increase in locomotor activity. These results are in general agreement with the data of Table I which show that compounds *Ia*, *IIa*, and *IIIa* are closely similar to *N*-methyl-α-5,9-dimethyl-6,7-benzomorphan with respect to times of onset and duration of action and thus probably are exhibiting a genuine analgesic effect, whereas *Ib* and *IIb* exhibit a delayed onset and prolonged duration of "analgesic action" which may, in fact, be attributed to depression rather than to true analgesia.

The prolonged depressant actions of *Ib* and *IIb* were also accompanied by severe and prolonged decreases in body temperature (Table III). The onset and duration of the hypothermia paralleled approximately the depressant actions. It is particularly interesting to note that only compounds *Ib* and *IIb* produced hypothermia and that the other compounds listed in Table I had no significant effect on body temperature.

The mechanisms by which *Ib* and *IIb* produce their prolonged effects remain to be elucidated. Since the alcohols (*Ia* and *IIa*) corresponding to *Ib* and *IIb* are devoid of both depressant and hypothermic activities, it is possible that the prolonged duration of pharmacological activity of *Ib* and *IIb* is due to alkylation, by the derived ethyleniminium ions, of specific sites in the CNS concerned with motor and temperature regulation. In order for this to occur, these compounds must enter the CNS: it is pertinent in this connection to note that *IIIb*, which will cyclize very rapidly to a quaternary ethyleniminium ion (14) probably incapable of entering the CNS, is devoid of pharmacological activity. Compounds *Ib* and *IIb* will be significantly less reactive (14) and thus may enter the CNS before cyclization to the ethyleniminium ion takes place. Alternatively, *Ib* and *IIb* may be slowly metabolized to an active product which is neither rapidly metabolized nor excreted and hence produce prolonged activity. McMahon *et al.* (15) have demonstrated, for example, that α-(−)-acetylmethadol is slowly metabolized to the active product α-(−)-noracetylmethadol the analgesic effects of which are fairly prolonged. However, the present work does not serve to distinguish either of these possibilities.

The pretreatment studies (Tables II and III, bottom columns) offer some evidence that the spectrum of activities of *N*-methyl-α,5,9-dimethyl-6,7-benzomorphan and *Ib* and *IIb* are different, since the depressant and hypothermic effects were not reduced by prior administration of the standard compound, as might have been expected had all three compounds acted at a common receptor site.

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*Keyphrases*

α-5,9-dimethyl-6,7-benzomorphans, *N*-substituted—synthesis
Pharmacological screening—5,9-dimethyl-6,7-benzomorphans
Analgesic activity
Depressant activity
Hypothermic activity

# Titration of Residual Moisture in Lyophilized Vials

By EDGAR E. THEIMER and JOSEPH J. PAVELEK

**Karl Fischer titration of residual water in lyophilized vials can be performed very simply, without specialized equipment, and gives results which are more reliable than loss-on-drying methods.**

IN THE determination of moisture, the Karl Fischer (K.F.) titrimetric method presents many advantages over the weight-loss-on-drying method. The literature abounds with comparative studies, most of which favor the K.F. method. Levy, Murtaugh, and Rosenblatt (1) titrated water in penicillin vials, assuming a zero blank. Wiberley (2) stressed the use of micro-equipment and introduced some improvements intended to broaden the use of the K.F. reagent. Burger and Polderman (3) used similar technique in some precise work on effect of lyophilizing conditions on residual water, using mannitol, *etc.* For lyophilized proteinaceous preparations, Sobel (4) suggested a colorimetric procedure, and Alajos (5) devised a special two-chambered vial for extraction of water and subsequent K.F. titration, to prevent direct contact of K.F. reagent with the protein.

Figure 1 depicts a vial containing a lyophilized mixture of 100 mg. of mannitol and 20 mg. of acetylcholine chloride.[1] It was desired to set up a specification limiting the water content of these vials in production batches. The primary aim of this study was to devise a simple quantitative test for water, of reasonable precision, without the use of specialized apparatus, and as free as possible from sources of error.

The first test method used involved drying in a vacuum oven to constant weight. Since it seemed likely that the rubber center seal might lose some weight during this treatment, similar blank vials were simultaneously treated and a correction applied. After further trials, it became evident that this correction value was variable, sometimes becoming as large as 0.6 mg. Also, it was found difficult to prevent weight gain between drying and weighing. Another difficulty was the need for microweighing technique in handling relatively large vials. In addition, there was some evidence that slight differences in drying temperature gave different results. In order to deal with these difficulties, various rather awkward dodges were devised, including weighing the vials while still hot, withdrawing and discarding the center seal, and pairing of samples with individual blank vials, but eventually a different water determination method was sought.

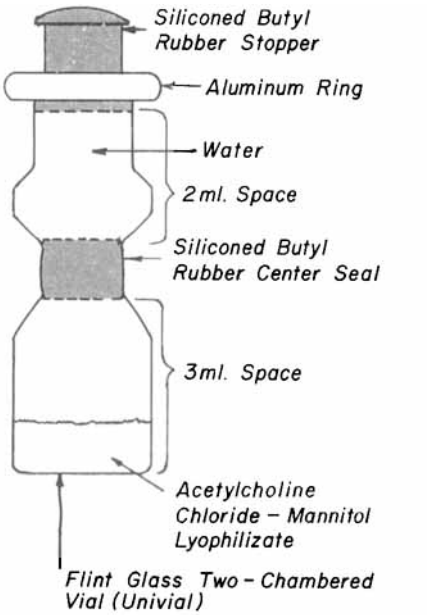


*Fig. 1—Vial containing lyophilized mixture of 100 mg. mannitol and 20 mg. acetylcholine chloride.*

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[1] Marketed as Miochol by Smith, Miller & Patch, Inc., New Brunswick, N. J.