# Action of Some Steroids on the Central Nervous System of the Mouse. II. Pharmacology

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The intravenous effects on mice of 142 pregnane derivatives and 26 other steroids were investigated; 67 of the pregnanes had hypnotic activity. Some of the steroids produced convulsions. Certain structure–activity relationships were apparent; these partly supported the conclusions of earlier workers. One compound, 3α-hydroxy-5β-pregnane-11,20-dione 3-phosphate disodium, tried in man as an intravenous anesthetic, produced an unpleasant paresthesia.

Under various experimental conditions some steroids are anesthetics,[1-3] and one of them, hydroxydione, has been used clinically.[4] This field was reviewed by Witzel,[5] who summarized results on 124 steroids. Later Overbeek[6] reported the effects of other steroids on the central nervous system, and some of them were anesthetic.

We present here the results of studies on mice of the hypnotic and other effects on the central nervous system of 168 steroids, 149 of which had not previously been tested in this way. The term hypnotic is preferred to anesthetic, because some steroids induce light sleep in mice without surgical anesthesia.

## Methods

Groups of 5–10 male fawn mice (GFF strain; body weights 16 to 22 g.) were injected intravenously with the steroids, usually presented at 1% concentration, either dissolved in water or suspended in physiological saline containing 0.4% Tween 80; occasionally, when the steroid had low toxicity or weak activity, higher concentrations were used. The suspensions were prepared by grinding the steroids with the vehicle in a glass tissue homogenizer. The compounds were administered in doses ranging from ineffective to hypnotic or lethal levels. When hypnosis resulted, the sleeping animals were placed in a cabinet maintained at 35°, and the times between beginning the injection and loss of the righting reflex ("induction time") and between loss and recovery of the righting reflex ("sleep time") were recorded. A group mean induction time of 0.1 min. indicates that all the mice were asleep at the end of injection.

For some steroids the intravenous $LD_{50}$ values were determined on groups of 5–15 mice; for others the amount available permitted the determination of only approximate values.

Figdor and co-workers[3] determined for each of their hypnotic steroids the $AD_{50}$, *i.e.*, the dose causing half the mice to lose their righting reflex. We preferred to determine the dose that induced sleep for 25 min. This duration was chosen because with group mean sleep times below 15 min. some of the mice did not sleep; with times over 35 min. the variance of the responses tended to be high. The 25-min. sleep-time dose and the corresponding induction time were determined graphically for each hypnotic steroid from the curves relating logarithms of doses to responses. For hypnotics toxic at doses below the 25-min. sleep dose the maximum measurable sleep time and the corresponding dose and induction time were usually recorded. (Steroids with such low therapeutic indices seemed unlikely to be of practical value.) In nontoxic doses certain steroids produced ataxia without loss of the righting reflex; this was regarded as mild hypnosis.

The steroids were synthesized by our colleagues, whose work is reported in the preceding paper.[7]

## Results and Discussion

The results for 142 pregnane compounds are given in Table I, where the steroids are classified according to the spatial arrangement of the hydrogen atom at C-5 and the substituents on C-11 and C-3.

Ten of the 63 5α-pregnane and 52 of the 76 5β-pregnane compounds produced hypnosis: one 5β-pregnane and four 5α-pregnane derivatives produced ataxia only. The three 5-unsaturated pregnane derivatives were inactive.

Convulsions were produced by 20 compounds, one of which also had hypnotic activity. Convulsant activity was usually shown by either a steroid alcohol or its ester, but not by both.

Thirteen 5-epimeric pairs of pregnane derivatives were tested. In four instances (**3**, **68**; **22**, **79**; **23**, **80**; and **53**, **131**) neither epimer was hypnotic: **3** was a convulsant, but not **68**. The acute toxicity of **53** was greater than that of its 5β-epimer. In five instances (**24**, **83**; **25**, **84**; **30**, **88**; **31**, **90**; and **56**, **137**) both epimers were hypnotics. The hypnotic doses of the 5α-steroids were similar to those of the corresponding 5β-steroids, but the acute toxicities of two esters of the same 5α-steroid (**24** and **25**) were greater than those of the 5β-epimer (**83** and **84**). In four instances (**4**, **69**; **21**, **78**; **51**, **130**; and **54**, **134**) only the 5β-epimer was hypnotic; **21** and **51** were convulsants. The water-soluble compounds **4** and **54** had acute toxicities similar to those of their 5β-epimers. Thus we encountered no 5α-steroid hypnotic with an inactive 5β-epimer.

**3-Hydroxypregnanone Derivatives.**—The 3-hydroxy-5ξ-pregnan-20-one series and their 11-oxo derivatives form a group of eight steroids. One of these, **64**, was as potent as any steroid we tested; its 11-oxo derivative (**88**) was only half as potent. The high hypnotic activity of **64** has been previously reported.[5a]

Comparison of **64** and **88** with their esters reveals some of the important effects of esterification; **66** and **67**, the succinate and phosphate of **64**, were somewhat less potent than the alcohol, whereas the corresponding acetate **65** was substantially less potent. Fifteen esters of **88** were studied; one of these, the succinate, has

(1) H. Selye, *Endocrinology*, **30**, 437 (1942).

(2) H. Selye and R. D. H. Heard, *Anesthesiology*, **4**, 36 (1943).

(3) S. K. Figdor, M. J. Kodet, B. M. Bloom, E. J. Agnello, S. Y. P'An, and G. D. Laubach, *J. Pharmacol. Exptl. Therap.*, **119**, 299 (1957).

(4) J. D. Robertson in "Recent Advances in Anesthesia and Analgesia," C. Langton Hewer, Ed., 9th Ed., J. and A. Churchill Ltd., London, 1963, pp. 30–78.

(5)(a) H. Witzel, *Z. Vitamin-, Hormon- Fermentforsch.*, **10**, 46 (1959); (b) H. Langecker and E. Busch, unpublished observations cited by Witzel.

(6) G. A. Overbeek, *Excerpta Med., Intern. Congr. Ser.*, **51**, 43 (1962).

(7) J. D. Cocker, J. Elks, P. J. May, F. A. Nee, G. H. Phillipps, and W. F. Wall, *J. Med. Chem.*, **8**, 417 (1965).

been independently reported to be a hypnotic.[6] The maleate **94**, the sulfate **95**, the phthalate **96**, the morpholinoacetate methiodide **100**, and the diethylaminoacetate ethiodide **99** had no hypnotic activity; indeed **99** caused convulsions. All of the other esters of **88** were less active than their parent steroid, some markedly so. Like the three esters of **64**, the acetate **89**, the succinate **90**, the phosphate **91**, the glutarate **92**, and the diglycolate **93** had longer induction times than the corresponding steroid alcohol, but the hypnotic amino esters **97**, **98**, and **101** had induction times approximately equal to or shorter than that of **88**. Compound **101** had unusual properties. It was the least active for which a 25-min. sleep dose could be determined, yet, unlike other weakly active steroids, it induced hypnosis of a satisfactory quality, with smooth induction and recovery.

Thus, we agree with the conclusions of Figdor and coworkers[3]: the 3-esters of hypnotic steroids are in general less potent than the free alcohols; the latter, especially the 3$\alpha$-ols, have short induction times; esterification, other than with amino acids, lengthens induction. They suggested that amino acid esters of steroids may enter the brain unhydrolyzed, adducing as evidence the high toxicity of such compounds; but **101** in our series had a low toxicity. It may be relevant that glycinates of antiinflammatory steroids have been recommended for their specific effect on the central nervous system.[8]

The 3$\alpha$-hydroxy steroid **30** and its succinate **31** had approximately the same hypnotic activity as each other and as their 5$\beta$-epimers **88** and **90**. Neither 3$\alpha$-hydroxy-5$\alpha$-pregnan-20-one (the 11-desoxy derivative of **30**) nor 3$\beta$-hydroxy-5$\beta$-pregnane-11,20-dione appear to have been tested for hypnotic properties, and they were unfortunately not available to us. However, we found that **103** (the 3$\alpha$-methyl derivative of 3$\beta$-hydroxy-5$\beta$-pregnane-11,20-dione) was inactive, whereas its 3-epimer **102** was hypnotic, with a short induction time. Though we have not usually recorded acute toxicity results for water-insoluble steroids, it was noted that **102** was much more toxic than **103**. The results with these compounds contrast with those of Langecker and Busch[5] for 17$\alpha$-ethynyl-3$\alpha$-methyl-5$\alpha$-androstane-3$\beta$,17$\beta$-diol: this was a hypnotic, yet its less toxic 3-epimer was a convulsant.

The 3$\beta$-hydroxy steroid **3** caused convulsions, and its succinate **4** was inactive; other derivatives of **3** (**5**–**12**) also had no hypnotic activity, and several of them were convulsants.

The 5$\beta$-epimer of **3** (**68**) had no hypnotic activity in sublethal doses; yet **69**, its succinate, was a fairly potent hypnotic. Similarly, although **32** was inactive, its succinate **33** was a weak hypnotic and, exceptionally, its phosphate **34** was even more active. Compound **68** has been reported by other workers as having hypnotic properties.[3] We did not control the particle size of our suspensions. Though this may explain the unusual apparent production of hypnotic activity by esterification, it could not explain two other disagreements between our results and those of Laubach's group.[3] First, we found the soluble succinate **69** much more hypnotic than they did; second, they reported **4** as hypnotic, though we have found it inactive. Notwithstanding the doubt as to the properties of **4**, **32**, and **68**, it is apparent that the most potent and rapidly acting compounds of this group are those with an unesterified 3$\alpha$-hydroxyl, whatever the configuration at C-5.

In some of this group of pregnanolone derivatives, the effects of 16-methylation have been studied; the results do not support Witzel's general conclusion that alkyl substituents diminish hypnotic activity.[5]

The 16$\alpha$-methyl derivative **72** was about half as potent as its parent steroid **64**. The induction time for **72** (4.1 min.) was one of the longest we recorded for steroids with a free 3-hydroxyl group; nevertheless its slightly more potent succinate **73** had a still longer induction time. 16$\alpha$-Methylation had different effects on the 11-oxo steroid **88**. The steroid alcohol and succinate **104** and **105** were more potent than **88** and **90**, although the phosphate **106** had the same hypnotic activity as **91**. 16$\beta$-Methylation of **88** (**107**) markedly increased its potency to that of the most potent steroid **64**. Likewise the succinate **108** was more potent than **90**, but not as potent as the succinate of **64**. 16,16-Dimethyl substitution on **88** and its succinate **90**, yielding **110** and **111**, respectively, had little effect on potency; **40**, the 16$\beta$-methyl derivative of **32**, was, like the latter, not a hypnotic, but the succinate **41** produced ataxia, as did the 16$\alpha$-methyl succinate ester **39**. The 17-epimer (**42**) of **41** produced convulsions.

Other substituents on the steroid nucleus found to result in loss of hypnotic activity were 12$\alpha$-acetoxyl (compare **70** with **64**; **71** with **65**), 17$\alpha$-hydroxyl or -acetoxyl (compare **45** and **46** with **33**; **113** with **88**), 17$\alpha$-hydroperoxyl (compare **74** with **64**; **112** with **88**), 16$\alpha$-hydroxyl (compare succinate esters **37** and **33**), and 16$\alpha$,17$\alpha$-epoxide (compare **38** with **33**).

The 20-ethylene ketal of **90** (**129**) was almost as potent a hypnotic as its parent, but it had an increased induction time and was about twice as toxic.

A 9(11)-ethylenic bond, present in **14** and **15**, appeared to introduce weak hypnotic activity into **3**, but in **77**, whose saturated parent **66** was highly active, the presence of hypnotic activity was doubtful: with such toxic compounds it is difficult to differentiate loss of the righting reflex and the prostration that precedes death. 16,17-Unsaturation (**35**) abolished the hypnotic activity of **33** and reduced activity in the 16-methyl steroid **109** (compare with **107**).

21-Benzylidene substitution (**125**) in **90** substantially increased the toxicity. Halogenation at C-21 (compare **126** with **88**; **127** and **128** with **89**) either decreased or abolished hypnotic activity. The 21-hydroxyl derivatives of the pregnanolones are discussed below.

**Pregnanedione Derivatives.**—5$\beta$-Pregnane-3,20-dione (**78**) was a potent hypnotic, but it had a long induction time; its 11-oxo and 11(12)-unsaturated derivatives **130** and **87** were less potent. 6$\alpha$-Methylation (**79**) abolished the hypnotic activity of **78**. 5$\alpha$-Pregnane-3,20-dione (**21**) and its 11-oxo derivative **51** caused convulsions. The former has been previously reported to be hypnotic, with much less activity than its 5$\beta$-epimer.[1,3] Compound **22**, the 6$\alpha$-methyl derivative of **21**, and **52**, the 1(2)-unsaturated derivative of **51**, were inactive.

**21-Hydroxypregnanedione Derivatives.**—21-Hydroxy-5$\beta$-pregnane-3,20-dione (**81**) had, within the

(8) J. Tamm and K.-D. Voigt, *Acta Endocrinol., Suppl.*, **54**, 1 (1960).

TABLE I

CENTRAL EFFECTS OF INTRAVENOUSLY ADMINISTERED PREGNANE COMPOUNDS ON MICE

| No. | Steroid | Activity[a] | Mean 25-min. sleep dose, mg./kg. | Induction time (min.) at 25-min. sleep dose | $LD_{50}$, mg./kg. |
|---|---|---|---|---|---|
| | 5α-Pregnanes | | | | |
| | 11-Unsubstituted, 3-unsubstituted | | | | |
| 1[b] | 5α-Pregnan-20-one | C | | | |
| | 11-Unsubstituted, 3-hydroxy | | | | |
| 2 | 5α-Pregnan-3β-ol 3-hemisuccinate sodium | 0 | | | 48 |
| 3[b] | 3β-Hydroxy-5α-pregnan-20-one | C | | | |
| 4 | 3β-Hydroxy-5α-pregnan-20-one 3-hemisuccinate sodium (34)[c] | 0 | | | *Ca.* 80 |
| 5 | 3β-Hydroxy-5α-pregn-16-en-20-one 3-hemisuccinate sodium | 0 | | | 90 |
| 6 | 3β-Hydroxy-5α-pregnane-12,20-dione 3-hemisuccinate sodium | C | | | 150 |
| 7 | 3β-Hydroxy-5α-pregn-16-ene-12,20-dione 3-hemisuccinate sodium | C | | | 250 |
| 8[b] | 3β-Hydroxy-16α-methoxy-5α-pregnane-12,20-dione | C | | | |
| 9[b] | 3β,16α-Dihydroxy-5α-pregnan-20-one | 0 | | | |
| 10 | 3β,16α-Dihydroxy-5α-pregnan-20-one 3,16-bis(hemisuccinate sodium) | C | | | *Ca.* 350 |
| 11 | 3β-Hydroxy-16α-methyl-5α-pregnan-20-one 3-hemisuccinate sodium | 0 | | | *Ca.* 50 |
| 12[b] | 17α-Hydroperoxy-3β-hydroxy-5α-pregnan-20-one | C | | | |
| 13 | 3β,21-Dihydroxy-5α-pregnan-20-one 3-hemisuccinate sodium 21-acetate (70) | (H) | | | 130 |
| 14[b] | 3β-Hydroxy-5α-pregn-9(11)-en-20-one | A | | | |
| 15 | 3β-Hydroxy-5α-pregn-9(11)-en-20-one 3-hemisuccinate sodium | (H) C | >200 | <13 | *Ca.* 250 |
| 16[b] | 3β,5-Dihydroxy-5α-pregna-7,9(11)-diene-20α-carboxylic acid sodium 3-acetate | 0 | | | >400 |
| 17[b] | 17α-Hydroperoxy-3β-hydroxy-16β-methyl-5α-pregn-9(11)-en-20-one | 0 | | | |
| 18 | 16α,17α-Epoxy-3β-hydroxy-16β-methyl-5α-pregn-9(11)-en-20-one | 0 | | | |
| 19 | 3β,17α-Dihydroxy-5α-pregn-9(11)-en-20-one 3-hemisuccinate sodium | 0 | | | 15 |
| 20 | 3β,17α,21-Trihydroxy-5α-pregn-9(11)-en-20-one 3-hemisuccinate sodium 21-acetate | 0 | | | 140 |
| | 11-Unsubstituted, 3-oxo | | | | |
| 21[b] | 5α-Pregnane-3,20-dione (43) | C | | | |
| 22 | 6α-Methyl-5α-pregnane-3,20-dione | 0 | | | |
| 23 | 17α,21-Dihydroxy-5α-pregnane-3,20-dione 21-hemisuccinate sodium | 0 | | | 320 |
| 24 | 21-Hydroxy-5α-pregnane-3,20-dione 21-hemisuccinate sodium (84) | H | 42 | >2 | 70 |
| 25 | 21-Hydroxy-5α-pregnane-3,20-dione 21-phosphate disodium | H | 64 | 2.6 | 170 |
| | 11-Oxo, 3-unsubstituted | | | | |
| 26[b] | 5α-Pregnane-11,20-dione | A | | | |
| 27[b] | 5α-Pregn-2-ene-11,20-dione | C | | | |
| 28 | 21-Hydroxy-5α-pregnane-11,20-dione | C | | | |
| 29 | 21-Hydroxy-5α-pregnane-11,20-dione 21-hemisuccinate sodium | H | 210 | 5.2 | <225 |
| | 11-Oxo, 3-hydroxy | | | | |
| 30[b] | 3α-Hydroxy-5α-pregnane-11,20-dione | H | 64 | 0.1 | |
| 31 | 3α-Hydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium | H | 64 | 3.7 | 310 |
| 32[b] | 3β-Hydroxy-5α-pregnane-11,20-dione | 0 | | | |
| 33 | 3β-Hydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium | H | 185 | 5.9 | 310 |
| 34 | 3β-Hydroxy-5α-pregnane-11,20-dione 3-phosphate disodium | H | 87 | 6.0 | 680 |
| 35 | 3β-Hydroxy-5α-pregn-16-ene-11,20-dione 3-hemisuccinate sodium | 0 | | | 210 |
| 36 | 3β,16α-Dihydroxy-5α-pregnane-11,20-dione | 0 | | | |
| 37 | 3β,16α-Dihydroxy-5α-pregnane-11,20-dione 3,16-bis(hemisuccinate sodium) | 0 | | | >1000 |
| 38 | 16α,17α-Epoxy-3β-hydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium | 0 | | | 320 |
| 39 | 3β-Hydroxy-16α-methyl-5α-pregnane-11,20-dione 3-hemisuccinate sodium | A | | | 300 |
| 40[b] | 3β-Hydroxy-16β-methyl-5α-pregnane-11,20-dione | 0 | | | |
| 41 | 3β-Hydroxy-16β-methyl-5α-pregnane-11,20-dione 3-hemisuccinate sodium | A | | | 175 |
| 42 | 3β-Hydroxy-16β-methyl-5α,17α-pregnane-11,20-dione 3-hemisuccinate sodium | C | | | 175 |
| 43 | 3β-Hydroxy-16-methyl-5α-pregn-16-ene-11,20-dione 3-hemisuccinate sodium | C | | | 225 |
| 44[b] | 17α-Hydroperoxy-3β-hydroxy-5α-pregnane-11,20-dione | C | | | |
| 45 | 3β,17α-Dihydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium | 0 | | | 445 |
| 46 | 3β,17α-Dihydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium 17-acetate | C | | | *Ca.* 300 |
| 47 | 3β-Hydroxy-17α-(2-tetrahydropyranyloxy)-5α-pregnane-11,20-dione | 0 | | | |
| 48 | 3β,21-Dihydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium 21-acetate (free-ol, 105) | 0 | | | 340 |
| 49 | 3β,17α,21-Trihydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium 21-acetate | 0 | | | >200 |
| 50 | 3β,17α,21-Trihydroxy-5α-pregnane-11,20-dione 3,21-bis(hemisuccinate sodium) | 0 | | | >1000 |
| | 11-Oxo, 3-oxo | | | | |
| 51[b] | 5α-Pregnane-3,11,20-trione | C | | | |
| 52 | 5α-Pregn-1-ene-3,11,20-trione | 0 | | | |
| 53 | 17α,21-Dihydroxy-5α-pregnane-3,11,20-trione 21-hemisuccinate sodium | 0 | | | 178 |
| 54 | 21-Hydroxy-5α-pregnane-3,11,20-trione 21-hemisuccinate sodium | 0 | | | 550 |

Table I (*Continued*)

| No. | Steroid | Activity[a] | Mean 25-min. sleep dose, mg./kg. | Induction time (min.) at 25-min. sleep dose | $LD_{50}$, mg./kg. |
|---|---|---|---|---|---|
| | Other 11-substituents | | | | |
| 55[b] | 11α-Hydroxy-5α-pregnane-3,20-dione | 0 | | | |
| 56[b] | 11α-Hydroxy-5α-pregnane-3,20-dione 11-acetate | H | 140 | 5.0 | |
| 57 | 3β,11β-Dihydroxy-5α-pregnan-20-one 3-hemisuccinate sodium | 0 | | | 170 |
| 58 | 3β,11β,17α-Trihydroxy-5α-pregnan-20-one 3-hemisuccinate sodium | 0 | | | 660 |
| 59 | 5α-Pregnane-3β, 11β, 20β-triol 3-hemisuccinate sodium | 0 | | | 40 |
| 60 | 11β-Hydroxy-5α-pregnane-3,20-dione | 0 | | | |
| 61 | 9α,11α-Epoxy-3β-hydroxy-5α-pregnan-20-one | C | | | |
| 62 | 9α,11α-Epoxy-3β-hydroxy-5α-pregnan-20-one 3-hemisuccinate sodium | C | | | *Ca.* 180 |
| 63 | 9β,11β-Epoxy-3β-hydroxy-5α-pregnan-20-one | 0 | | | |
| | 5β-Pregnanes | | | | |
| | 11-Unsubstituted, 3-hydroxy | | | | |
| 64[b] | 3α-Hydroxy-5β-pregnan-20-one (37) | H | 27 | 0.9 | |
| 65[b] | 3α-Hydroxy-5β-pregnan-20-one 3-acetate | H | 71 | 9.5 | |
| 66 | 3α-Hydroxy-5β-pregnan-20-one 3-hemisuccinate sodium (38) | H | 33 | 3.1 | 87 |
| 67 | 3α-Hydroxy-5β-pregnan-20-one 3-phosphate disodium | H | 42 | 2.1 | 260 |
| 68[b] | 3β-Hydroxy-5β-pregnan-20-one (35) | 0 | | | |
| 69 | 3β-Hydroxy-5β-pregnan-20-one 3-hemisuccinate sodium (36) | H | *Ca.* 50 | 7.0 | <100 |
| 70[b] | 3α,12α-Dihydroxy-5β-pregnan-20-one 12-acetate | 0 | | | |
| 71[b] | 3α,12α-Dihydroxy-5β-pregnan-20-one 3,12-diacetate | 0 | | | |
| 72 | 3α-Hydroxy-16α-methyl-5β-pregnan-20-one | H | 49 | 4.1 | |
| 73 | 3α-Hydroxy-16α-methyl-5β-pregnan-20-one 3-hemisuccinate sodium | H | 43 | 6.3 | 75 |
| 74[b] | 17α-Hydroperoxy-3α-hydroxy-5β-pregnan-20-one | 0 | | | |
| 75 | 3α,21-Dihydroxy-5β-pregnan-20-one (79) | H | 37 | 1.9 | |
| 76[b] | 3α,21-Dihydroxy-5β-pregnan-20-one 21-acetate | H | 76 | 0.9 | |
| 77 | 3α-Hydroxy-5β-pregn-9(11)-en-20-one 3-hemisuccinate sodium | H? | | | *Ca.* 100 |
| | 11-Unsubstituted, 3-oxo | | | | |
| 78[b] | 5β-Pregnane-3,20-dione (44) | H | 52 | 3.2 | |
| 79 | 6α-Methyl-5β-pregnane-3,20-dione | 0 | | | |
| 80 | 17α,21-Dihydroxy-5β-pregnane-3,20-dione 21-hemisuccinate sodium (108) | 0 | | | 390 |
| 81[b] | 21-Hydroxy-5β-pregnane-3,20-dione (85) | H | 33 | 5.9 | |
| 82[b] | 21-Hydroxy-5β-pregnane-3,20-dione 21-acetate | H | 90 | 4.8 | |
| 83[b] | 21-Hydroxy-5β-pregnane-3,20-dione 21-hemisuccinate sodium (hydroxydione, 86) | H | 44 | 2.2 | 205 |
| 84 | 21-Hydroxy-5β-pregnane-3,20-dione 21-phosphate disodium | H | 54 | 3.6 | 390 |
| 85 | 21-Hydroxy-5β-pregnane-3,20-dione $P^1,P^2$-bis(21-pyrophosphate) disodium | (H) | | | 175 |
| 86 | 21-Hydroxy-5β-pregnane-3,20-dione 21-benzylphosphate sodium | C | | | *Ca.* 140 |
| 87[b] | 5β-Pregn-11-ene-3,20-dione | H | 100 | 5.2 | |
| | 11-Oxo, 3-hydroxy | | | | |
| 88[b] | 3α-Hydroxy-5β-pregnane-11,20-dione | H | 59 | 0.3 | |
| 89[b] | 3α-Hydroxy-5β-pregnane-11,20-dione 3-acetate | H | 90 | 1.8 | |
| 90 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-hemisuccinate sodium | H | 67 | 1.8 | 350 |
| 91 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-phosphate disodium | H | 73 | 2.0 | 465 |
| 92 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-hemiglutarate sodium | H | 95 | 1.4 | 330 |
| 93 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-hemidiglycolate sodium | H | 85 | 2.3 | 325 |
| 94 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-hemimaleate sodium | 0 | | | >800 |
| 95 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-sulfate sodium | 0 | | | >600 |
| 96 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-hemiphthalate sodium | 0 | | | 125 |
| 97 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-aminoacetate hydrochloride | H | 86 | 0.3 | 75 |
| 98 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-diethylaminoacetate hydrochloride | H | 74 | 0.4 | 125 |
| 99 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-diethylaminoacetate ethiodide | C | | | 13 |
| 100 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-morpholinoacetate methiodide | 0 | | | 25 |
| 101 | 3α-Hydroxy-5β-pregnane-11,20-dione 3-hemi-N-acetyl-L-glutamate sodium | H | 420 | 0.2 | 625 |
| 102 | 3α-Hydroxy-3β-methyl-5β-pregnane-11,20-dione | (H) | >30 | 0.1 | *Ca.* 40 |
| 103 | 3β-Hydroxy-3α-methyl-5β-pregnane-11,20-dione | 0 | | | *Ca.* 150 |
| 104[b] | 3α-Hydroxy-16α-methyl-5β-pregnane-11,20-dione | H | 48 | 0.5 | |
| 105 | 3α-Hydroxy-16α-methyl-5β-pregnane-11,20-dione 3-hemisuccinate sodium | H | 47 | 7.1 | 130 |
| 106 | 3α-Hydroxy-16α-methyl-5β-pregnane-11,20-dione 3-phosphate disodium | H | 70 | 5.8 | 260 |
| 107 | 3α-Hydroxy-16β-methyl-5β-pregnane-11,20-dione | H | 28 | 0.8 | |
| 108 | 3α-Hydroxy-16β-methyl-5β-pregnane-11,20-dione 3-hemisuccinate sodium | H | 43 | 2.6 | 160 |
| 109 | 3α-Hydroxy-16-methyl-5β-pregn-16-ene-11,20-dione | (H) | >200 | <2.0 | |
| 110 | 3α-Hydroxy-16,16-dimethyl-5β-pregnane-11,20-dione | H | 62 | 0.4 | |
| 111 | 3α-Hydroxy-16,16-dimethyl-5β-pregnane-11,20-dione 3-hemisuccinate sodium | H | 78 | 2.8 | 130 |
| 112[b] | 17α-Hydroperoxy-3α-hydroxy-5β-pregnane-11,20-dione | 0 | | | |

TABLE I (*Continued*)

| No. | Steroid | Activity[a] | Mean 25-min. sleep dose, mg./kg. | Induction time (min.) at 25-min. sleep dose | $LD_{50}$, mg./kg. |
|---|---|---|---|---|---|
| 113[b] | 3α,17α-Dihydroxy-5β-pregnane-11,20-dione | 0 | | | |
| 114[b] | 3α,21-Dihydroxy-5β-pregnane-11,20-dione | 0 | | | |
| 115 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 3-acetate | H | 250 | 5.0 | |
| 116[b] | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 21-acetate | H | 97 | 0.1 | |
| 117 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 21-hemisuccinate sodium | H | 185 | 5.9 | *Ca.* 600 |
| 118 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 21-phosphate disodium | H | 200 | 6.5 | >900 |
| 119 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 21-*t*-butylacetate | H | 78 | 0.5 | |
| 120 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 21-methanesulfonate | A | | | |
| 121[b] | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 3,21-diacetate | (H) | >125 | <7.0 | |
| 122 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 3-acetate 21-methanesulfonate | 0 | | | |
| 123 | 3α,21-Dihydroxy-5β-pregnane-11,20-dione 3-hemisuccinate sodium 21-acetate | H | 152 | 7.6 | 500 |
| 124 | 3α-Hydroxy-21-mercapto-5β-pregnane-11,20-dione 21-acetate | H | 35 | 0.5 | |
| 125 | 21-Benzylidene-3α-hydroxy-5β-pregnane-11,20-dione 3-hemisuccinate sodium | H | <100 | >4.0 | 120 |
| 126 | 21-Fluoro-3α-hydroxy-5β-pregnane-11,20-dione | (H) | >100 | 0.1 | |
| 127 | 21-Chloro-3α-hydroxy-5β-pregnane-11,20-dione 3-acetate | 0 | | | |
| 128 | 3α-Hydroxy-21-iodo-5β-pregnane-11,20-dione 3-acetate | 0 | | | |
| 129 | 3α-Hydroxy-5β-pregnane-11,20-dione 20-ethylene ketal 3-hemisuccinate sodium | H | 75 | 7.0 | 150 |
| | 11-Oxo, 3-oxo | | | | |
| 130[b] | 5β-Pregnane-3,11,20-trione | H | 78 | 3.0 | |
| 131 | 17α, 21-Dihydroxy-5β-pregnane-3,11,20-trione 21-hemisuccinate sodium | 0 | | | >800 |
| 132[b] | 21-Hydroxy-5β-pregnane-3,11,20-trione | 0 | | | |
| 133[b] | 21-Hydroxy-5β-pregnane-3,11,20-trione 21-acetate | 0 | | | |
| 134 | 21-Hydroxy-5β-pregnane-3,11,20-trione 21-hemisuccinate sodium | H | 150 | | 500 |
| | Other 11-substituents | | | | |
| 135[b] | 3α,11α-Dihydroxy-5β-pregnan-20-one | (H) | >50 | <0.7 | |
| 136[b] | 3α,11α-Dihydroxy-5β-pregnan-20-one 11-acetate | (H) | >100 | 0.1 | |
| 137[b] | 11α-Hydroxy-5β-pregnane-3,20-dione 11-acetate | H | 140 | 4.0 | |
| 138 | 3α,11β-Dihydroxy-5β-pregnan-20-one 3-hemisuccinate sodium | H | 250 | 5.5 | 375 |
| 139 | 11α,12α-Epoxy-5β-pregnane-3,20-dione | 0 | | | |
| | 5-Unsaturated Pregnanes | | | | |
| 140 | 3β, 17α-Dihydroxy-19-norpregna-5,7,9-trien-20-one 3-hemisuccinate sodium | 0 | | | *Ca.* 380 |
| 141[b] | 3β-Hydroxypregn-5-en-20-one (45) | 0 | | | |
| 142 | 3β, 21-Dihydroxypregn-5-en-20-one 3-hemisuccinate sodium 21-acetate (94) | 0 | | | 49 |

[a] 0 = inactive, H = hypnotic, (H) = hypnotic at toxic levels with indeterminate 25-min. sleep dose, H? = hypnotic activity doubtful, A = ataxia only, C = convulsant. [b] The preparation of these compounds is described in the literature. For the others, see the preceding paper.[5] [c] Numbers in brackets are those assigned to the steroids in Table III of ref. 5.

limits of experimental error, the same hypnotic activity as the most potent of our series, **64**.[3] Its induction time was lengthy: such steroids as this and the 3-oxo steroids above demonstrate that, though an unesterified 3-hydroxyl usually confers rapid induction, this property is not shared by the 21-hydroxyl or 3-oxo groups.

At least some of the hypnotic activity of 3-oxo steroids may result from their metabolic transformation to active 3-hydroxy steroids.[3] Langecker[9] showed that hydroxydione is metabolized in human subjects to 3α-hydroxy steroids; 3β-hydroxy steroids were not found.

Compound **82**, the 21-acetate of **81**, was only one third as active as the parent alcohol, whereas the succinate (hydroxydione **83**) was almost equally active. We found **84**, the phosphate of **81**, to be almost as active and about half as toxic as hydroxydione; Irmscher[10] claimed that the phosphate is more active than the succinate.

The bis steroid pyrophosphate **85** was almost inactive and markedly toxic; the benzyl phosphate **86** caused convulsions. The properties of other esters of **81** have been reported.[3]

(9) H. Langecker, *Acta Endocrinol.*, **30**, 369 (1959).
(10) K. Irmscher, *Chem. Ind.* (London), 1035 (1961).

The 5α-epimers of hydroxydione and of its phosphate analog **84** (**24** and **25**, respectively) were also hypnotics, of similar potency but greater toxicity. We did not test the free alcohol, 21-hydroxy-5α-pregnane-3,20-dione, but it has been reported as less potent than the 5β-epimer.[3]

The 11-oxo derivative of **81** (**132**) was inactive; so was **133**, the acetate of **132**. The succinate **134** was hypnotic, though it was much less potent than hydroxydione; **54**, the 5α-epimer of **134**, was inactive.

Compound **29**, the 3-unsubstituted derivative of **54**, had a weak but distinct hypnotic action. This was surprising, for it refuted Witzel's proposition that hypnotic activity depends on the presence of hydroxyl or ketone groups on both ends of the steroid nucleus.[5] Similarly **26**, which produced ataxia, was an exception.

The 17α-hydroxy derivatives of 21-hydroxypregnane-dione and -trione succinates were inactive: compare **80** with **83**, **23** with **24**, **131** with **134**, and **53** with **54**. Langecker and Busch found that **80** injected intraperitoneally was a weak hypnotic, at a dose that we found too high for intravenous administration.[5]

**3,21-Dihydroxypregnanone Derivatives.**—Isomerism at C-3 and C-5 allows four possible structures for 3,21-dihydroxypregnan-20-one. We have examined

Table II

Other Steroids Lacking Hypnotic Activity

| No. | Compound |
|---|---|
| | Androstane Compounds |
| 143 | 5α-Androstan-3β-ol 3-hemisuccinate sodium |
| 144 | 3β-Hydroxy-5α-androstan-11-one 3-hemisuccinate sodium |
| 145 | 3β-Hydroxy-5α-androstane-11,17-dione 3-hemisuccinate sodium |
| 146[a] | 3β-Hydroxyandrost-5-en-17-one 3-hemisuccinate sodium |
| | D-Homoandrostane Compounds |
| 147 | 3β-Hydroxy-D-homo-5α-androstane-11,17a-dione[b] |
| 148 | 3β,17α-Dihydroxy-17β-methyl-D-homo-5α-androstane-11,17a-dione |
| 149 | 3β,17α-Dihydroxy-16β,17β-dimethyl-D-homo-5α-androst-9(11)-en-17a-one |
| 150 | 3β,17α-Dihydroxy-16α,17β-dimethyl-16β-fluoro-D-homo-5α-androstane-11,17a-dione 3-acetate |
| 151[a] | 3α,17α-Dihydroxy-17β-methyl-D-homo-5β-androstane-11,17a-dione[b] |
| 152[a] | 3α,17aα-Dihydroxy-17aβ-methyl-D-homo-5β-androstane-11,17-dione |
| 153[a] | 3α,17aβ-Dihydroxy-17aα-methyl-D-homo-5β-androstane-11,17-dione |
| 154 | 3α,17aβ-Dihydroxy-17aα-methyl-D-homo-5β-androstane-11,17-dione 3-hemisuccinate sodium[b] |
| | Etianic Acid Compounds |
| 155[a] | Sodium 3-oxo-5α-etianate |
| 156[a] | Sodium 3β,17α-dihydroxy-11-oxo-5α-etianate |
| 157[a] | Sodium 3-oxo-5β-etianate |
| 158[a] | Sodium 3α,12α-dihydroxy-5β-etianate |
| 159[a] | Sodium 3-oxoeti-4-enate |
| 160[a] | Sodium 17α-hydroxy-3-oxoeti-4-enate |
| | Miscellaneous Compounds |
| 161 | Estra-5,7,9-triene-3β,17β-diol 3,17-bis(hemisuccinate sodium) |
| 162 | 17α-Methylestra-5,7,9-triene-3β,17β-diol 3-hemisuccinate sodium |
| 163 | 3β-Hydroxyestra-5,7,9-trien-17-one 3-hemisuccinate sodium |
| 164 | 16α-Acetyl-3β-hydroxy-16β-methyl-5α-androst-9(11)-en-17-one 3-acetate |
| 165 | 16β-Acetyl-3β-hydroxy-16α-methyl-5α-androst-9(11)-en-17-one[b] |
| 166 | 16β-Acetyl-3β-hydroxy-16α-methyl-5α-androstane-11,17-dione 3-acetate |
| 167 | Sodium 16ξ-acetyl-3β-hydroxy-16ξ-methyl-16,17-seco-5α-androst-9(11)-en-17-oate[b] |
| 168 | Methyl 16ξ-acetyl-3β-hydroxy-16ξ-methyl-16,17-seco-5α-androst-9(11)-en-17-oate |

[a] The preparation of these compounds is described in the literature; for the others, see the preceding paper. [b] Convulsant.

some of these and some of their 11-oxo derivatives, as both free alcohols and esters.

3α,21-Dihydroxy-5β-pregnan-20-one (**75**) was almost as hypnotic as the corresponding 3-oxo and 21-deoxy compounds **81** and **64**; its induction time was between those of **81** and **64**. The 21-acetate **76** was only half as potent as the parent steroid, but its induction time was also halved. The 3-epimer of **75**, and some of its esters, have been reported as less hypnotic than **75**.[3]

Compound **114** (the 11-oxo derivative of **75**) was inactive, like its 3-oxo counterpart **132**. The 3-acetate of **114** (**115**) was weakly active, with a long induction time. The 21-acetate **116** had a short induction time and was much more potent, not as potent as its 11-deoxy derivative **76**, but more so than its 3-oxo analog **133**. The 3,21-diacetate of **114** (**121**) was too toxic for adequate testing; however, it was clearly hypnotic and had a long induction time, as did the corresponding 3-succinate 21-acetate **123**.

The 21-succinate **117** and the 21-phosphate **118** of **114** were weakly hypnotic and, despite their unesterified 3α-hydroxyl groups, their induction times were long. However, the more potent water-insoluble 21-ester **119** had, like the 21-acetate, a short induction time, indicating that it must have dissolved rapidly in the bloodstream. It is possible that **114** is too polar to pass readily into the brain, and that its 21-succinate and -phosphate have little hypnotic activity because they too are polar or because they are readily hydrolyzed in the bloodstream; the less polar, less readily hydrolyzed 21-esters may pass as such into the brain, there to act by virtue of their unesterified 3α-hydroxyl groups.

The 21-methanesulfonate of **114** (**120**) produced only ataxia, and the 3-acetate 21-methanesulfonate **122** was inactive. Thus not all the water-insoluble 21-esters of **114** were hypnotics.

The introduction of a sulfur atom into **116** (**124**) considerably increased its potency and gave it peculiar properties. Mice dosed with **124** appeared to experience an intense skin irritation after recovering the righting reflex: they scratched themselves vigorously, and some circled madly inside their cages. Some had inadequate control of their hind limbs for the first 10 min. after awakening.

3β,21-Dihydroxy-5α-pregnan-20-one has apparently not been tested though its 21-succinate and 3-acetate 21-succinate have been reported; we too studied the 3-succinate 21-acetate (**13**), which was toxic but distinctly hypnotic.[3]

3β,21-Dihydroxy-5α-pregnane-11,20-dione has been reported[1,2] to be weakly hypnotic; under our conditions its 3-succinate 21-acetate **48** and two esters of its 17α-hydroxyl analog (**49** and **50**) were inactive.

**11-Substitution.**—Twenty-six pairs of compounds in our series illustrate the effects of 11-oxo substitution, which will now be summarized.

In three instances (**1**, **26**; **3**, **32**; and **10**, **37**) the 11-unsubstituted steroid only and in two instances (**12**, **44** and **21**, **51**) both the 11-unsubstituted and the 11-oxo steroid were convulsant; no 11-oxo steroid caused convulsions unless its 11-deoxy derivative also did. In only three of the pairs (**1**, **26**; **4**, **33**; and **11**, **39**) had the

11-oxo steroid a greater hypnotic activity than the 11-unsubstituted steroid. Some of these compounds have just been described as exceptional in other respects, **26** having no substituent on C-3, **4** being previously reported as a hypnotic,[3] and **33** being an ester whose free alcohol is inactive.

In two of the pairs (**72**, **104** and **73**, **105**) which were 16$\alpha$-methyl steroids, the hypnotic activities were equal and in twelve (**13**, **48**; **24**, **54**; **64**, **88**; **65**, **89**; **66**, **90**; **67**, **91**; **75**, **114**; **76**, **116**; **78**, **130**; **81**, **132**; **82**, **133**; and **83**, **134**) the 11-unsubstituted steroid was the more active: indeed, five of the 11-oxo steroids (**48**, **54**, **114**, **132**, and **133**) were inactive. Induction times tended to be shorter with the 11-oxo steroids.

In all but one (**23**, **53**) of the twelve pairs for which $LD_{50}$ values were recorded, the toxicity of the 11-oxo steroid was lower, often much lower, than that of the corresponding 11-unsubstituted compound. The therapeutic indices ($LD_{50}$/25-min. sleep dose) of 11-oxo steroids tended to be higher, as they were for three of the four directly comparable pairs.

11-Hydroxyl or -acetoxyl and 9,11- or 11,12-epoxide substitution diminished or abolished any hypnotic activity of the parent steroids. Water-soluble 11-hydroxy steroids were less toxic than their 11-deoxy counterparts.

**Further Studies.**—3$\alpha$-Hydroxy-5$\beta$-pregnane-11,20-dione 3-phosphate disodium (**91**) was considered promising as an intravenous anesthetic. It formed stable aqueous solutions, had a high therapeutic index, and did not produce thrombophlebitis in experimental animals as hydroxydione did.

Two *Cynomolgus* monkeys injected intravenously with 145 and 165 mg. of **91**/kg. of body weight slept for about 130 min. (induction time 4 to 5 min.). After a dose of 385 mg./kg. another monkey slept for 5 hr.; recovery was rapid. A fourth monkey received a dose of 540 mg./kg.; it slept for more than 8 hr., and the next day it had fully recovered. Two cats injected intraperitoneally with 150 mg./kg. became surgically anesthetized in 20 or 30 min.; after a further 60 min. the sleeping cats were given intravenous doses repeatedly over a period of 105 min. The cats survived total doses of 800 or 900 mg./kg.

This steroid was tried clinically by Dr. A. H. Galley, who has kindly allowed us to describe his results. Intravenous doses of 1.0 to 1.5 g. produced sleep in adult patients. In all of them, shortly after a first injection of as little as 50 mg., an extremely unpleasant paresthesia developed. "Prickling" or "pins and needles" began in the head and extended to the trunk and legs; it was worst in the buttocks. The paresthesia ceased spontaneously after a few minutes and did not recur with subsequent doses. This symptom, also found by Robertson,[4] was considered sufficiently serious to preclude further use of the steroid. Its cause is unknown, but it may be related to the ability of the steroid to release erythrocyte potassium into the plasma,[11] or to the pyrogenicity of its parent steroid (though **81** is also pyrogenic and hydroxydione, its succinate, is not).[6,12]

**Other Steroids.**—The 26 other steroids we tested are listed in Table II. Some produced convulsions, but none had hypnotic activity. Selye found that one of them, 3$\beta$-hydroxyandrost-5-en-17-one 3-hemisuccinate sodium (dehydroepiandrosterone succinate) was hypnotic in the partially hepatectomized rat.[1]

(11) R. M. Atkinson, I. G. MacGregor, M. A. Pratt, and E. G. Tomich, *Biochem. Pharmacol.*, **12**, 631 (1963).

(12)(a) A. Kappas, W. Soybel, D. K. Fukushima, and T. F. Gallagher, *Trans. Assoc. Am. Physicians*, **72**, 54 (1959); (b) A. Kappas, W. Soybel, P. Glickman, and D. K. Fukushima, *A.M.A. Arch. Internal Med.*, **105**, 701 (1960).

# Synthesis of *cis*- and *trans*-2-Phenoxycyclopropylamines and Related Compounds

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In continuing the study of MAO inhibitors in these laboratories, the two 2-phenoxycyclopropylamines were synthesized and found to be potent compounds. From the various intermediates, derivatives were prepared to explore their biological potentials. Also, several substituted aryloxycyclopropylamines were synthesized to determine the relationship between chemical constitution and pharmacological activity.

In 1959, Tedeschi and co-workers[1] announced the discovery of a potent nonhydrazine monoamine oxidase inhibitor, SKF (*trans*) 385. This compound, 2-phenylcyclopropylamine hydrochloride,[2a] had been synthesized some years earlier by Burger and Yost[2b] in connection with a study of cyclized sympathomimetic amines. A preliminary report[3] indicated that the compound is more rapid in its action, of shorter duration, effective at smaller doses, and relatively free of the side effects exhibited by the hydrazine monoamine oxidase inhibitors used for the treatment of depression. However, since its clinical evaluation is complicated by its strong amphetamine-like action,[4] we decided to synthesize some related compounds which might retain the desired pharmacological activity of the new drug without this side effect. For this purpose, we synthesized *cis*- and *trans*-2-phenoxycyclopropylamine and some of its derivatives,[5] as well as several substituted aryloxycyclopropylamines. In the interim, this compound was

(1) R. E. Tedeschi, D. H. Tedeschi, L. Cook, P. A. Mattis, and E. J. Fellows, presented at the 43rd Federation Meeting, April 13–17, 1959, Atlantic City, N. J.

(2) (a) Parnate, tranylcypromine, Smith Kline and French Laboratories, Inc.; (b) A. Burger and W. L. Yost, *J. Am. Chem. Soc.*, **70**, 2198 (1948).

(3) F. Lemaire, *Am. J. Psychiat.*, **117**, 249 (1960).

(4) V. J. Kincross-Wright, *Ann. N. Y. Acad. Sci.*, **80**, 840 (1959).

(5) J. Finkelstein, F. A. Smith, and J. Lee, Belgian Patent 613,910 (Feb. 21, 1961).