

# Esters of Nipecotic and Isonipecotic Acids as Potential Anticonvulsants

A. MICHAEL CRIDER \*\*, TERENCE T. TITA \*, J. D. WOOD ‡, and CHRISTINE N. HINKO \*

Received September 24, 1981, from the \*College of Pharmacy, University of Toledo, Toledo, OH 43606 and the †Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan. Accepted for publication January 11, 1982.

**Abstract** □ A variety of esters of nipecotic and isonipecotic acids were synthesized and evaluated for anticonvulsant activity. The ester group was varied in terms of lipophilicity and reactivity toward hydrolysis. The esters were screened against seizures induced by electroshock, pentylenetetrazol, and the putative  $\gamma$ -aminobutyric acid antagonist, bicuculline. The most significant activity was demonstrated by the *p*-nitrophenyl esters of nipecotic and isonipecotic acids against bicuculline-induced seizures. Esters of nipecotic acid were tested for *in vitro* inhibition of  $\gamma$ -aminobutyric acid and L-proline uptakes into mouse whole brain minislices. The *p*-nitrophenyl, *n*-octyl, and succinimidyl esters were the most potent inhibitors of  $\gamma$ -aminobutyric acid uptake. The uptake of  $\gamma$ -aminobutyric acid by the ester derivatives appeared to involve specific and nonspecific mechanisms.

**Keyphrases** □ Anticonvulsants—potential, esters of nipecotic and isonipecotic acids, mice □ Nipecotic acid—esters, isonipecotic acid, potential anticonvulsants, mice □ Isonipecotic acid—esters, nipecotic acid, potential anticonvulsants, mice

Over the past several years evidence has been accumulated to implicate  $\gamma$ -aminobutyric acid as a major central nervous system (CNS) inhibitory neurotransmitter (1, 2). Impairment of  $\gamma$ -aminobutyric acid neurotransmission may cause a variety of neurological disorders such as epilepsy and certain psychiatric conditions (2, 3). As a result, considerable research has been directed toward the search for compounds that act as agonists, uptake inhibitors, or inhibitors of the metabolizing enzymes of  $\gamma$ -aminobutyric acid (4). The hope has been that agents that potentiate  $\gamma$ -aminobutyric acid neurotransmission could be potentially useful anticonvulsants.

## BACKGROUND

( $\pm$ )-Nipecotic acid (Ia) was shown to be a potent inhibitor of neuronal  $\gamma$ -aminobutyric acid uptake into rat cerebral cortex (5) and mouse whole brain minislices (6). Additional studies found that the *R*-(-) enantiomer was more active as a  $\gamma$ -aminobutyric acid uptake inhibitor than the *S*-(+) enantiomer (7).

Due to its polarity, nipecotic acid does not readily penetrate the blood-brain barrier (8). However, it was previously shown (9) that (+)-ethyl nipecotate, when administered intraperitoneally, protected mice against audiogenic seizures. Furthermore, it was found (10) that (-)-ethyl nipecotate raised the threshold for convulsions induced by electroshock in mice. These same investigators also demonstrated that (-)- and (+)-ethyl nipecotate significantly elevated the threshold for clonic convulsions induced by pentylenetetrazol. (-)-Nipecotic acid had no effect in these anticonvulsant tests when administered intraperitoneally.

Recently, it was found that (*R*)-ethyl nipecotate given intramuscularly in mice resulted in a dose-dependent increase in  $\gamma$ -aminobutyric acid levels in the synaptosomes (11). The speculation was that such an increase in  $\gamma$ -aminobutyric acid levels in the nerve endings possibly could bring about an anticonvulsant effect.

During the final stages of this work, the anticonvulsant activity of a series of esters of the  $\gamma$ -aminobutyric acid agonist, isoguvacine, was reported (12). Most of the esters exhibited weak anticonvulsant activity against seizures induced by bicuculline or isoniazid. However, a good correlation existed between the onset of electroshock activity and the rates of *in vitro* enzymatic hydrolysis.

Isonipecotic acid (Ib) was shown to be a potent and specific  $\gamma$ -aminobutyric acid agonist in the [ $^3$ H] $\gamma$ -aminobutyric acid-binding assay procedure (13, 14). As in the case of nipecotic acid, isonipecotic acid was also too polar to penetrate the blood-brain barrier.

The purpose of this investigation was to prepare and evaluate the anticonvulsant activity of ester derivatives of nipecotic and isonipecotic acids. The ester group was varied in terms of lipophilicity and reactivity toward hydrolysis. Since the amino acids (Ia and Ib) are too polar to penetrate the CNS, it was anticipated that the ester derivatives would enter the brain and be hydrolyzed to yield the respective amino acids.

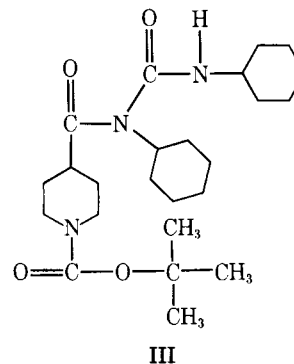
The synthesis and anticonvulsant activity of the ester derivatives of nipecotic and isonipecotic acids are described in the present report. Additionally,  $\gamma$ -aminobutyric acid and L-proline uptake studies into mice whole brain minislices by the ester derivatives of nipecotic acid are reported.

## RESULTS AND DISCUSSION

**Chemistry**—Esterification of carboxylic acids can be accomplished by the use of dehydrating agents such as dicyclohexylcarbodiimide, provided an acid catalyst is present (15). Although *N*-hydroxysuccinimide and *p*-nitrophenol are acidic enough to furnish a proton, the aliphatic alcohols are not. As a result, the reaction between 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (IIb) and *n*-hexanol initially gave a thermally unstable *O*-acylisourea (16), which rearranged to the more stable *N*-acylurea (III). The alkyl esters (IVa-f) were successfully synthesized by Fischer esterification of the piperidine carboxylic acids (Ia and Ib) with an appropriate alcohol in the presence of dry hydrogen chloride. The remaining esters were conveniently prepared by standard procedures (Scheme I). The physical properties of these derivatives are given in Tables I and II.

**Biological Testing**—In initial investigations<sup>1</sup>, three of the piperidinyl esters showed some activity against the subcutaneous pentylenetetrazol seizure test. The octyl ester (IVf) exhibited an ED<sub>50</sub> of 165 mg/kg in the subcutaneous pentylenetetrazol seizure test at the time of peak effect (4 hr). However, IVf showed considerable toxicity (TD<sub>50</sub> = 178 mg/kg) when evaluated in the rotarod test at 4 hr after administration of the compound. Compounds IIa and VIa protected one out of four animals when administered 0.5 hr before pentylenetetrazol in doses of 600 and 300 mg/kg, respectively. Although no neurotoxicity was reported for IIa, compound VIa produced deficiency in rotarod performance in two out of four animals when administered in a dose of 300 mg/kg.

The subcutaneous pentylenetetrazol test was repeated in the laboratory with three of the esters, which were administered 1 hr prior to the con-



<sup>1</sup> All compounds were tested for anticonvulsant activity by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Health, Bethesda, MD 20225.

valant by subcutaneous injection in an attempt to reduce peripheral hydrolysis that might occur with the intraperitoneal route. The results are shown in Table III. Sodium phenobarbital (25 mg/kg sc), the reference compound, completely protected the mice tested from clonic and tonic seizure. Esters VIIIa, VIIIb, and VIa, in 150-mg/kg doses, all significantly lengthened the onset time for myoclonic activity as compared to the saline-treated control animals. The succinimidyl ester (VIa) was most active, protecting three out of the eight animals from clonic convulsions. All three esters protected the animals tested from tonic seizure and death. These results, however, are difficult to interpret since only 2 of the 16 control animals died after exhibiting tonic convulsions, and pentylene-tetrazol is most commonly used for its ability to produce threshold or minimal (clonic) seizures (17). Neurotoxicity was not observed with any of the three esters at the 150-mg/kg dose.

These three compounds were also evaluated for their ability to protect against bicuculline-induced convulsions. The results are shown in Table IV. Each of the three esters at a 150-mg/kg dose significantly prolonged the onset time of clonic seizures when administered by subcutaneous injection. The *p*-nitrophenyl ester (VIIIa) was most active against bicuculline, protecting four out of eight animals from any clonic activity and completely protecting all eight mice from tonic seizure and death. These results are especially significant when compared to the controls where bicuculline produced clonic and tonic seizures and resulted in fatality in 100% of the saline-treated mice. The *p*-nitrophenyl ester (VIIIb), although not as effective as VIIIa, protected three out of eight mice from clonic seizure and seven out of eight from tonic convulsions and death. The succinimidyl ester (VIa), which showed activity against pentylene-tetrazol produced no protection from myoclonus and only minimal protection (two out of eight mice) from tonic seizures. Four out of eight animals, however, were protected from fatality. Additional experiments were performed in an attempt to maximize the anticonvulsant activity of VIa. However, increasing the dose to 225 mg/kg and varying the pre-treatment time did not alter its effects. The reference compound, diazepam (10 mg/kg ip), prevented bicuculline-induced seizure activity in all animals tested.

The results of the uptake studies of the esters of nipecotic acid, with respect to  $\gamma$ -aminobutyric acid and L-proline into mouse whole brain minislices, are given in Table V. Although all esters showed appreciable inhibition of  $\gamma$ -aminobutyric acid uptake, IVc, VIa, and VIIIa were the most potent. In order for the esters to exhibit inhibition of  $\gamma$ -aminobutyric acid uptake, hydrolysis to the parent amino acid (Ia) is necessary. Thus, it is not surprising that VIa inhibited  $\gamma$ -aminobutyric acid uptake in a manner identical to nipecotic acid. NMR analysis of VIa indicates that the ester is hydrolyzed very rapidly to the parent amino acid. ( $\pm$ )-ethyl nipecotate demonstrated moderate inhibition (47%) as contrasted with an earlier report (7) indicating that the (-)-isomer was inactive.

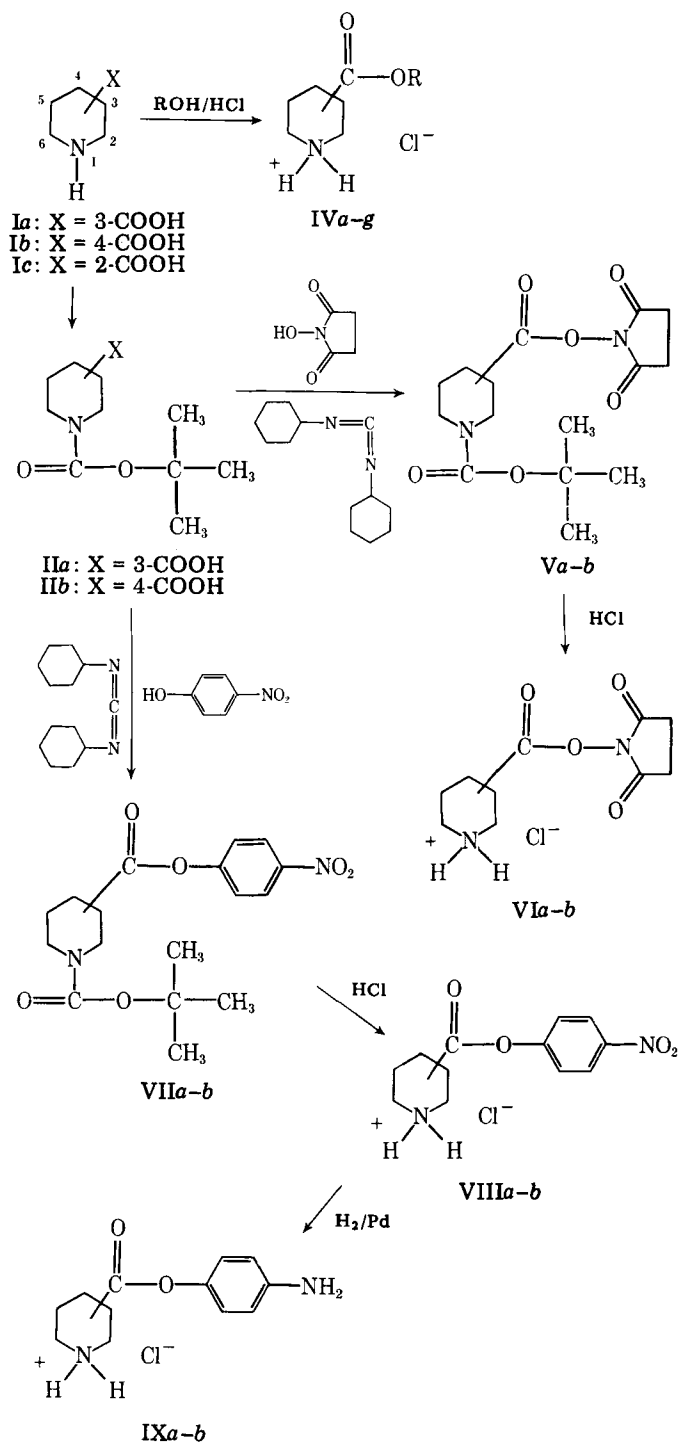
In all probability, the octyl ester (IVc) is acting as a nonspecific inhibitor of  $\gamma$ -aminobutyric acid uptake. In comparison with Ia, IVc is a much more potent inhibitor of L-proline (99 versus 21% inhibition). The octyl ester (IVc) may well be exerting its inhibitory activity by a nonspecific detergent-like effect. Compound IVc probably interrupts all types of membrane functions, causing  $\gamma$ -aminobutyric acid and L-proline inhibition.

Compound VIIIa presents a different case than IVc. Although VIIIa seems to exhibit nonspecificity, as evidenced by its 70% inhibition of L-proline uptake at  $1 \times 10^{-3}$  M, it does not inhibit L-proline as potently as IVc. Dilution of VIIIa does not decrease the  $\gamma$ -aminobutyric acid uptake inhibition as drastically as that for IVc.

One possible explanation for these results is that the observed effects of IVc and VIIIa are the summation of two different types of inhibition, specific and nonspecific. The nonspecific effect is due to the intact ester, while the specific effect is caused by hydrolysis of IVc and VIIIa to nipecotic acid. Support for this hypothesis can be obtained by noting that at  $1 \times 10^{-3}$  M, both IVc and VIIIa are more potent than Ia in the  $\gamma$ -aminobutyric acid uptake assay. Furthermore, pipecolic acid is a much weaker inhibitor of  $\gamma$ -aminobutyric acid uptake than Ia (Table V). However, the octyl esters (IVc and IVg) of the amino acids were equal in potency as  $\gamma$ -aminobutyric acid uptake inhibitors. These results suggest that IVc and IVg are not hydrolyzed under the assay conditions, and that their inhibition is due to a nonspecific cationic detergent effect.

If this hypothesis is correct, the nonspecific mechanism appears to assume a greater role in the case of IVc than VIIIa. Support for this can be gathered by noting that VIIIa did not inhibit L-proline to the extent of IVc. Furthermore, the inhibition of L-proline by VIIIa was always much less than that of IVc.

Further studies are in progress to define more accurately the anti-



Scheme I

convulsant activity of VIIIa as well as to determine the effect of VIIIa on the  $\gamma$ -aminobutyric acid content of nerve endings using a synaptosomal model.

## EXPERIMENTAL<sup>2</sup>

**Nipecotic Acid (Ia)**—Compound Ia was prepared by the method of Freifelder (18) in 62% yield, mp 258–260° [lit. (19) mp 260–261°].

**1-(tert-Butyloxycarbonyl)piperidine-3-carboxylic Acid (IIa)**—A solution of Ia (10.0 g, 0.077 mole) in 50 ml of water was treated with tri-

<sup>2</sup> Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were recorded as potassium bromide pellets with a Perkin-Elmer 137 spectrophotometer. NMR spectra were recorded on a Varian EM 360A spectrometer. Chemical shifts are reported in parts per million ( $\delta$ ) relative to tetramethylsilane (1%) or in the case of D<sub>2</sub>O (1%) sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

**Table I—Physical Properties of *n*-Alkyl Piperidinecarboxylate Hydrochlorides**

| Compound | R                                                | Substituent<br>Ring<br>Position | Melting Point | Yield, % <sup>a</sup> | Formula                                           | Analysis, %                  |                        |
|----------|--------------------------------------------------|---------------------------------|---------------|-----------------------|---------------------------------------------------|------------------------------|------------------------|
|          |                                                  |                                 |               |                       |                                                   | Calc.                        | Found                  |
| IVa      | —(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> | 3                               | 51–53°        | 39                    | C <sub>10</sub> H <sub>20</sub> ClNO <sub>2</sub> | C 54.13<br>H 9.11<br>N 6.32  | 54.36<br>9.04<br>6.12  |
| IVb      | —(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub> | 3                               | 32–34°        | 34                    | C <sub>12</sub> H <sub>24</sub> ClNO <sub>2</sub> | C 57.69<br>H 9.70<br>N 5.61  | 57.64<br>9.76<br>5.86  |
| IVc      | —(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub> | 3                               | 43–45°        | 57                    | C <sub>14</sub> H <sub>28</sub> ClNO <sub>2</sub> | C 60.51<br>H 10.18<br>N 5.61 | 60.31<br>10.12<br>5.86 |
| IVd      | —(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> | 4                               | 113–115°      | 66                    | C <sub>10</sub> H <sub>20</sub> ClNO <sub>2</sub> | C 54.16<br>H 9.11<br>N 6.32  | 54.31<br>9.27<br>6.18  |
| IVe      | —(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub> | 4                               | 73–75°        | 50                    | C <sub>12</sub> H <sub>24</sub> ClNO <sub>2</sub> | C 57.69<br>H 9.70<br>N 5.61  | 57.61<br>9.54<br>5.38  |
| IVf      | —(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub> | 4                               | 75–77°        | 60                    | C <sub>14</sub> H <sub>28</sub> ClNO <sub>2</sub> | C 60.51<br>H 10.18<br>N 5.04 | 60.75<br>10.32<br>4.91 |
| IVg      | —(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub> | 2                               | 128–129°      | 82                    | C <sub>14</sub> H <sub>28</sub> ClNO <sub>2</sub> | C 60.51<br>H 10.18<br>N 5.04 | 59.83<br>10.33<br>5.06 |

<sup>a</sup> The yield of analytically pure hydrochloride after one recrystallization from ethyl acetate, except IVf which was recrystallized from chloroform-ethyl acetate.

ethylamine (11.9 g, 0.116 mole) followed by the addition of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (21.0 g, 0.085 mole) in 50 ml of acetone. After stirring overnight, the reaction mixture was treated with 250 ml of an equal ethyl acetate–water mixture. The water layer was separated and the ethyl acetate phase was washed with an additional 100 ml of water. The combined aqueous phases were washed with 100 ml of ethyl acetate and acidified with cold 1 *N* HCl to pH 2. Upon cooling, a white solid precipitated from solution. Recrystallization from absolute ethanol–water gave 14.4 g (81%) of IIa, mp 149–151°; IR (KBr): 1750 (C=O, acid) and 1680 (C=O, carbamate) cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 1.45

[s, 9H, C(CH<sub>3</sub>)<sub>3</sub>], 1.65 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH), 2.30–3.20 (broad m, CHNCH and CH overlap), 4.0 (m, 2H, CHNCH), and 10.1 (broad s, 1H, COOH) ppm.

Anal.—Calc. for C<sub>11</sub>H<sub>19</sub>NO<sub>4</sub>: C, 57.61; H, 8.37; N, 6.11. Found: C, 57.80; H, 8.32; N, 5.99.

1-(*tert*-Butyloxycarbonyl)piperidine-4-carboxylic Acid (IIb)—Compound IIb was prepared, as described for IIa, from Ib (10.0 g, 0.077 mole), triethylamine (11.9 g, 0.116 mole) and 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (21.0 g, 0.085 mole). Recrystallization of the product from absolute ethanol–water yielded 13.3 g (75%) of ana-

**Table II—Physical Properties of Piperidine Derivatives**

| Compound | Substituent<br>Ring<br>Position | Melting Point | Yield, % | Recrystal-<br>lization<br>Solvent <sup>a</sup> | Formula                                                         | Analysis, %                  |                        |
|----------|---------------------------------|---------------|----------|------------------------------------------------|-----------------------------------------------------------------|------------------------------|------------------------|
|          |                                 |               |          |                                                |                                                                 | Calc.                        | Found                  |
| IIa      | 3                               | 149–151°      | 81       | A                                              | C <sub>11</sub> H <sub>19</sub> NO <sub>4</sub>                 | C 57.61<br>H 8.37<br>N 6.11  | 57.80<br>8.32<br>5.99  |
| IIb      | 4                               | 135–137°      | 75       | A                                              | C <sub>11</sub> H <sub>19</sub> NO <sub>4</sub>                 | C 57.61<br>H 8.37<br>N 6.11  | 57.62<br>8.32<br>6.21  |
| Va       | 3                               | 125–127°      | 61       | B                                              | C <sub>15</sub> H <sub>22</sub> N <sub>2</sub> O <sub>6</sub>   | C 55.19<br>H 6.81<br>N 8.58  | 55.19<br>6.85<br>8.76  |
| Vb       | 4                               | 139–141°      | 67       | B                                              | C <sub>15</sub> H <sub>22</sub> NO <sub>6</sub>                 | C 55.19<br>H 6.81<br>N 8.58  | 54.95<br>6.71<br>8.82  |
| VIa      | 3                               | 168–170°      | 69       | B                                              | C <sub>10</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>4</sub> | C 45.71<br>H 5.77<br>N 10.66 | 45.95<br>5.94<br>10.83 |
| VIb      | 4                               | 205–207°      | 62       | B                                              | C <sub>10</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>4</sub> | C 45.71<br>H 5.77<br>N 10.66 | 45.83<br>5.61<br>10.95 |
| VIIa     | 3                               | 73–75°        | 59       | C                                              | C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O <sub>6</sub>   | C 58.27<br>H 6.34<br>N 8.00  | 58.33<br>6.50<br>8.16  |
| VIIb     | 4                               | 91–93°        | 60       | B                                              | C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O <sub>6</sub>   | C 58.27<br>H 6.34<br>N 8.00  | 58.29<br>6.24<br>8.19  |
| VIIIa    | 3                               | 160–162°      | 60       | B                                              | C <sub>12</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>4</sub> | C 50.26<br>H 5.28<br>N 9.77  | 50.39<br>5.30<br>9.92  |
| VIIIb    | 4                               | 215–217°      | 77       | A                                              | C <sub>12</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>4</sub> | C 50.26<br>H 5.28<br>N 9.77  | 50.03<br>5.09<br>10.01 |
| IXa      | 3                               | 217–219°      | 73       | D                                              | C <sub>12</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>2</sub> | C 56.13<br>H 6.69<br>N 10.91 | 55.96<br>6.62<br>10.77 |
| IXb      | 4                               | 237–239°      | 66       | D                                              | C <sub>12</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>2</sub> | C 56.13<br>H 6.69<br>N 10.91 | 55.90<br>6.51<br>10.78 |

<sup>a</sup> (A) ethanol–water; (B) absolute ethanol; (C) ethanol–hexane; and (D) 95% ethanol.

Table III—Antagonism of Pentylene-tetrazol-Induced Convulsions by Esters of Nipecotic Acid

| Effect                                             | Saline       | Pretreatment <sup>a</sup><br>Sodium Phenobarbital<br>(25 mg/kg sc) | VIIIa<br>(150 mg/kg sc) | VIIIb<br>(150 mg/kg sc) | VIa<br>(150 mg/kg sc)   |
|----------------------------------------------------|--------------|--------------------------------------------------------------------|-------------------------|-------------------------|-------------------------|
| Onset of myoclonus <sup>b</sup> , min (Mean ± SEM) | 8.50 ± 0.5   | —                                                                  | 19.7 ± 0.7 <sup>c</sup> | 21.5 ± 3.8 <sup>c</sup> | 18.2 ± 1.9 <sup>c</sup> |
| Protected, %                                       | 0 (0/16)     | 100 (4/4) <sup>c</sup>                                             | 12.5 (1/8)              | 14.3 (1/7)              | 37.5 (3/8) <sup>d</sup> |
| Onset of tonic seizure, min                        | 20; 25       | —                                                                  | —                       | —                       | —                       |
| Protected, %                                       | 87.5 (14/16) | 100 (4/4)                                                          | 100 (8/8)               | 100 (7/7)               | 100 (8/8)               |
| Time of death, min                                 | 20; 60       | —                                                                  | —                       | —                       | —                       |
| Protected, %                                       | 87.5 (14/16) | 100 (4/4)                                                          | 100 (8/8)               | 100 (8/8)               | 100 (8/8)               |

<sup>a</sup> Pretreatments were administered 1 hr before injection of pentylenetetrazol (85 mg/kg sc). <sup>b</sup> Onset of myoclonus was defined as one episode of clonic activity persisting for at least 5 sec. <sup>c</sup> Significantly different from saline controls. ( $p < 0.01$ ). <sup>d</sup>  $p < 0.05$ .

Table IV—Antagonism of Bicuculline-Induced Convulsions by Esters of Nipecotic Acid

| Effect                                             | Saline    | Pretreatment <sup>a</sup><br>Diazepam <sup>b</sup><br>(10 mg/kg ip) | VIIIa<br>(150 mg/kg sc) | VIIIb<br>(150 mg/kg sc) | VIa<br>(150 mg/kg sc)   |
|----------------------------------------------------|-----------|---------------------------------------------------------------------|-------------------------|-------------------------|-------------------------|
| Onset of myoclonus <sup>c</sup> , min (Mean ± SEM) | 5.5 ± 0.3 | —                                                                   | 20.3 ± 2.8 <sup>d</sup> | 17.6 ± 3.0 <sup>d</sup> | 11.0 ± 2.5 <sup>d</sup> |
| Protected, %                                       | 0 (0/23)  | 100 (4/4)                                                           | 50 (4/8) <sup>d</sup>   | 37.5 (3/8) <sup>e</sup> | 0 (0/8)                 |
| Onset of tonic seizure, min (Mean ± SEM)           | 7.7 ± 0.4 | —                                                                   | —                       | 22.0                    | 11.6 ± 1.5 <sup>d</sup> |
| Protected, %                                       | 0 (0/23)  | 100 (4/4)                                                           | 100 (8/8) <sup>e</sup>  | 87.5 (7/8) <sup>e</sup> | 25 (2/8)                |
| Time of death, min                                 | 9.2 ± 1.3 | —                                                                   | —                       | 22.0                    | 15.7 ± 2.5 <sup>f</sup> |
| Protected, %                                       | 0 (0/23)  | 100 (4/4)                                                           | 100 (8/8) <sup>d</sup>  | 87.5 (7/8) <sup>d</sup> | 50 (4/8) <sup>d</sup>   |

<sup>a</sup> Pretreatments were administered 1 hr before injection of bicuculline (3 mg/kg sc). <sup>b</sup> Diazepam completely protected the mice from seizures as compared to the solvent for diazepam (40% propylene glycol–10% ethanol). <sup>c</sup> Onset of myoclonus was defined as one episode of clonic activity persisting for at least 5 sec. <sup>d</sup> Significantly different from saline controls ( $p < 0.01$ ). <sup>e</sup>  $p < 0.05$ . <sup>f</sup> This mean is based on  $n = 3$ , with the fourth animal dying ~2 hr postinjection of bicuculline.

lytically pure product, mp 135–137°; IR (KBr): 1750 (C=O, acid) and 1680 (C=O, carbamate)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ):  $\delta$  1.40 [s, 9H,  $\text{C}(\text{CH}_3)_3$ ], 1.80 (m, 4H,  $\text{CH}_2\text{CHCH}_2$ ), 2.60 (m, 3H, CHNCH and CH overlap), 4.0 (m, 2H, CHNCH), and 10.2 (broad s, 1H, COOH) ppm.

Anal.—Calc. for  $\text{C}_{11}\text{H}_{19}\text{NO}_4$ : C, 56.61; H, 8.37; N, 6.11. Found: C, 56.62; H, 8.32; N, 6.21.

N,N'-Dicyclohexyl-N-(1-tert-butyloxycarbonyl-4-piperidinocarbonyl)urea (III)—A solution of IIb (2.50 g, 0.011 mole) and *n*-hexanol (1.10 g, 0.011 mole) in 155 ml of acetonitrile was treated in one portion with 2.30 g (0.011 mole) of dicyclohexylcarbodiimide. The reaction mixture was stirred overnight, filtered, and the filtrate was concentrated under reduced pressure. The resulting oil was triturated with diethyl ether to afford after recrystallization from hexane 1.30 g (28%) of white solid, mp 166–168°; IR (KBr): 3350 (NH), 1700 (C=O, amide) and 1670 (C=O, carbamate and urea)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ):  $\delta$  1.30 [s, 9H,  $\text{C}(\text{CH}_3)_3$ ], 1.40–1.80 (broad m, 26H,  $\text{CH}_2\text{NCH}_2$  and cyclohexyl overlap), 2.20–2.60 (broad m, 3H, CHNCH and CHCO overlap), 4.0 (m, 2H, CHNCH), and 6.15 (d, 1H, NH) ppm.

Anal.—Calc. for  $\text{C}_{24}\text{H}_{41}\text{N}_3\text{O}_4$ : C, 66.16; H, 9.50; N, 9.65. Found: C, 65.91; H, 9.49; N, 9.44.

*n*-Alkyl Piperidinecarboxylate Hydrochlorides (IVa–g)—The synthesis of *n*-butyl 4-piperidinecarboxylate hydrochloride (IVd) was representative of the general method. A suspension of isonipecotic acid in 20 ml of *n*-butanol was saturated with dry hydrogen chloride and refluxed for 2 hr. The resulting solution was cooled, diluted with ether, and washed with four 50-ml portions of water. The aqueous phase was washed with 50 ml of ether, basified with sodium bicarbonate to pH 8, and extracted with four 50-ml portions of chloroform. The combined chloroform layer was dried (sodium sulfate) and evaporated to give a colorless oil. The oil was dissolved in anhydrous chloroform and was saturated with hydrogen chloride at 0°. Recrystallization of the hydrochloride from ethyl acetate gave 2.3 g (67%) of white crystalline solid, mp 113–115°; IR (KBr): 2500 ( $\text{NH}_2^+$ ), 1760 (C=O), and 1225 (C–O)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ):  $\delta$  0.90 (t, 3H,  $\text{CH}_3$ ), 1.50 (m, 4H,  $\text{CH}_2\text{CH}_2$ ), 2.15 (t, 4H,  $\text{CH}_2\text{CHCH}_2$ ), 2.50 (m, 1H, CH), 3.20 (m, 4H,  $\text{CH}_2\text{NH}_2^+\text{CH}_2$ ), 4.1 (t, 2H,  $\text{OCH}_2$ ), and 8.50–9.30 (broad s, 1H,  $\text{NH}_2^+$ ).

Anal.—Calc. for  $\text{C}_{10}\text{H}_{20}\text{ClNO}_2$ : C, 54.16; H, 9.11; N, 6.32. Found: C, 54.31; H, 9.27; N, 6.18.

**Preparation of tert-Butyloxycarbonyl Esters Va–b and VIIa–b**—The synthesis of *p*-nitrophenyl-1-(tert-butyloxycarbonyl)-4-piperidinecarboxylate (VIIb) is representative of the general procedure. A mixture of IIb (2.5 g, 0.011 mole) and *p*-nitrophenol (1.50 g, 0.011 mole) in 115 ml of acetonitrile was treated with dicyclohexylcarbodiimide (2.30 g, 0.011 mole). Immediately, a white precipitate of dicyclohexylurea formed. Stirring was continued overnight and the precipitated dicyclohexylurea was filtered, and the filtrate was concentrated. Recrystallization of the solid from absolute ethanol gave 2.30 g (60%) of white crystalline solid, mp 91–93°; IR (KBr): 1790 (C=O, ester) and 1700 (C=O,

carbamate)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ):  $\delta$  1.40 [s, 9H,  $\text{C}(\text{CH}_3)_3$ ], 1.80 (m, 4H,  $\text{CH}_2\text{CHCH}_2$ ), 2.80 (m, 3H, CHNCH and CH), 4.0 (m, 2H, CHNCH), 7.20–8.20 (m, 4H, ArH).

Anal.—Calc. for  $\text{C}_{17}\text{H}_{22}\text{NO}_6$ : C, 58.27; H, 6.34; N, 8.00. Found: C, 58.29; H, 6.24; N, 8.19.

**Removal of the tert-Butyloxycarbonyl Group from Va–b and VIIa–b**—*p*-Nitrophenyl 4-piperidinecarboxylate Hydrochloride (VIIIb)—Synthesis of VIIIb is typical of the general method. A solution of VIIb (2.70 g, 0.008 mole) in 100 ml of chloroform was saturated with hydrogen chloride at 0–5° for 1 hr. The solvent was evaporated and the resulting solid was recrystallized from ethanol–water to afford 1.70 g (78%) of a white crystalline solid, mp 215–217°; IR (KBr): 2500 ( $\text{NH}_2^+$ ), 1750 (C=O, ester), and 1180 (C–O)  $\text{cm}^{-1}$ ; NMR ( $\text{D}_2\text{O}$ ):  $\delta$  2.45 (m, 4H,  $\text{CH}_2\text{CHCH}_2$ ), 3.10–3.65 (broad m, 5H,  $\text{CH}_2\text{NCH}_2$  and CH overlap), and 7.45–8.35 (m, 4H, ArH).

Anal.—Calc. for  $\text{C}_{12}\text{H}_{15}\text{ClN}_2\text{O}_4$ : C, 50.26; H, 5.28; N, 9.77. Found: C, 50.03; H, 5.09; N, 10.01.

*p*-Aminophenyl 3-Piperidinecarboxylate Hydrochloride (IXa)—A suspension of VIIa (1.40 g, 0.0049 mole) and 0.2 g of 10% palladium on carbon in 250 ml of ethanol was hydrogenated on a hydrogenator<sup>3</sup> at an initial pressure of 3 atm. After 1 hr, the theoretical amount of hydrogen had been absorbed. The mixture was warmed gently to dissolve the product, filtered, and evaporated under reduced pressure. The solid product was recrystallized twice from ethanol to yield 0.92 g (73%) of a brown crystalline solid, mp 217–219°; IR (KBr): 3550 and 3450 ( $\text{NH}_2$ ), 2500 ( $\text{NH}_2^+$ ), 1780 (C=O, ester), and 1220 (C–O)  $\text{cm}^{-1}$ ; NMR ( $\text{D}_2\text{O}$ ):  $\delta$  1.90 (m, 4H,  $\text{CH}_2\text{CH}_2\text{CH}$ ), 2.70–3.70 (broad m, 5H,  $\text{CH}_2\text{NH}_2^+\text{CH}_2\text{CH}$ ) and 6.85 (s, 4H, ArH).

Anal.—Calc. for  $\text{C}_{12}\text{H}_{17}\text{ClN}_2\text{O}_2$ : C, 56.13; H, 6.69; N, 10.91. Found: C, 55.96; H, 6.62; N, 10.77.

*p*-Aminophenyl 4-Piperidinecarboxylate Hydrochloride (IXb)—Compound IXb was prepared from VIIIb (1.50 g, 0.005 mole), 0.2 g of 10% palladium on carbon in 250 ml of ethanol in the same manner as described for the synthesis of IXa. Recrystallization of the product from ethanol gave 0.89 g (66%) of analytically pure product, mp 237–239°; IR (KBr): 3550 and 3400 ( $\text{NH}_2$ ), 2500 ( $\text{NH}_2^+$ ), 1750 (C=O, ester), and 1190 (C–O)  $\text{cm}^{-1}$ ; NMR ( $\text{D}_2\text{O}$ ):  $\delta$  2.20 (m, 4H,  $\text{CH}_2\text{CHCH}_2$ ), 2.80–3.50 (broad m, 5H,  $\text{CH}_2\text{NH}_2^+\text{CH}_2$  and CH overlap) and 6.90 (s, 4H, ArH).

Anal.—Calc. for  $\text{C}_{12}\text{H}_{17}\text{ClN}_2\text{O}_2$ : C, 56.13; H, 6.69; N, 10.91. Found: C, 55.90; H, 6.51; N, 10.78.

**Biological Testing**—Initial evaluation for anticonvulsant activity was done by an antiepileptic drug development program<sup>1</sup> using the test systems as previously described (19). The testing included the maximal electroshock seizure test, the subcutaneous pentylenetetrazol seizure threshold test, and the rotarod test to evaluate neurotoxicity. In these

<sup>3</sup> Parr hydrogenator.

**Table V—Effect of Piperidine Derivatives on the Uptakes of  $\gamma$ -Aminobutyric Acid <sup>a</sup> and L-Proline <sup>b</sup>**

| Inhibitor                  | Concentration of Inhibitor, mM | Uptake System | Uptake, pmole/g/min <sup>c</sup> |                 |                             |
|----------------------------|--------------------------------|---------------|----------------------------------|-----------------|-----------------------------|
|                            |                                |               | Control                          | Inhibitor       | Inhibition, %               |
| Ia                         | 1.0                            | GABA          | 112.9 $\pm$ 6.7                  | 4.1 $\pm$ 0.3   | 96.3 $\pm$ 0.2              |
|                            | 0.2                            |               | 126.2 $\pm$ 5.8                  | 13.7 $\pm$ 0.6  | 89.0 $\pm$ 0.7              |
|                            | 0.05                           |               | 134.0 $\pm$ 2.7                  | 33.4 $\pm$ 1.6  | 75.1 $\pm$ 0.9              |
|                            | 0.02                           |               | 112.3 $\pm$ 8.7                  | 40.2 $\pm$ 2.1  | 63.8 $\pm$ 1.4              |
|                            | 1.0                            | Proline       | 47.2 $\pm$ 1.4                   | 37.4 $\pm$ 1.9  | 21.0 $\pm$ 2.9              |
| Ic                         | 0.2                            | GABA          | 45.6 $\pm$ 1.2                   | 40.2 $\pm$ 2.2  | 11.7 $\pm$ 5.1              |
|                            | 1.0                            |               | 117.8 $\pm$ 2.2                  | 80.7 $\pm$ 1.7  | 32.6 $\pm$ 0.8              |
|                            | 0.2                            |               | 111.6 $\pm$ 7.0                  | 95.0 $\pm$ 3.9  | 14.2 $\pm$ 2.8              |
| ( $\pm$ )-Ethyl nipecotate | 1.0                            | GABA          | 112.6 $\pm$ 5.2                  | 62.3 $\pm$ 3.2  | 47.4 $\pm$ 1.6              |
| IVa                        | 1.0                            | GABA          | 112.7 $\pm$ 4.9                  | 46.5 $\pm$ 1.9  | 59.7 $\pm$ 1.3              |
| IVb                        | 1.0                            | GABA          | 112.6 $\pm$ 4.3                  | 53.2 $\pm$ 0.7  | 52.7 $\pm$ 1.6              |
| IVc                        | 1.0                            | GABA          | 112.7 $\pm$ 4.2                  | 0.1 $\pm$ 0.1   | 99.7 $\pm$ 0.1              |
| IVg                        | 0.2                            | GABA          | 128.8 $\pm$ 8.7                  | 66.4 $\pm$ 2.7  | 47.8 $\pm$ 2.6              |
|                            | 0.05                           |               | 136.6 $\pm$ 6.4                  | 114.6 $\pm$ 4.8 | 16.0 $\pm$ 2.5              |
|                            | 1.0                            |               | 36.5 $\pm$ 2.4                   | 0.5 $\pm$ 0.2   | 98.8 $\pm$ 0.5              |
|                            | 0.2                            |               | 45.9 $\pm$ 1.5                   | 13.3 $\pm$ 1.0  | 70.9 $\pm$ 2.4              |
|                            | 1.0                            | Proline       | 116.5 $\pm$ 2.6                  | 0.2 $\pm$ 0.1   | 99.8 $\pm$ 0.1              |
|                            | 0.2                            |               | 107.6 $\pm$ 4.6                  | 50.6 $\pm$ 3.3  | 53.1 $\pm$ 1.6              |
|                            | 0.05                           |               | 94.3 $\pm$ 4.9                   | 73.0 $\pm$ 3.3  | 22.4 $\pm$ 1.4              |
| VIa                        | 1.0                            | GABA          | 119.0 $\pm$ 2.4                  | 4.3 $\pm$ 0.3   | 96.4 $\pm$ 0.3              |
| VIIIa                      | 0.2                            |               | 115.8 $\pm$ 5.8                  | 11.8 $\pm$ 0.8  | 89.7 $\pm$ 0.6              |
|                            | 0.05                           |               | 123.5 $\pm$ 5.2                  | 30.9 $\pm$ 1.2  | 74.9 $\pm$ 0.9              |
|                            | 1.0                            |               | 138.9 $\pm$ 5.5                  | 1.3 $\pm$ 0.1   | 99.0 $\pm$ 0.1              |
|                            | 0.5                            |               | 154.1 $\pm$ 6.0                  | 6.2 $\pm$ 0.4   | 95.8 $\pm$ 0.3 <sup>d</sup> |
|                            | 0.2                            | Proline       | 162.1 $\pm$ 4.1                  | 20.0 $\pm$ 1.2  | 87.6 $\pm$ 0.8              |
|                            | 0.05                           |               | 154.0 $\pm$ 6.6                  | 62.4 $\pm$ 6.0  | 59.7 $\pm$ 2.7              |
|                            | 1.0                            |               | 45.1 $\pm$ 0.6                   | 13.5 $\pm$ 0.8  | 70.0 $\pm$ 1.8              |
| IXa                        | 0.5                            |               | 45.3 $\pm$ 1.5                   | 16.3 $\pm$ 1.1  | 63.7 $\pm$ 2.8 <sup>d</sup> |
|                            | 0.2                            | GABA          | 45.9 $\pm$ 1.7                   | 23.7 $\pm$ 1.6  | 47.4 $\pm$ 3.9              |
|                            | 0.05                           |               | 44.1 $\pm$ 1.6                   | 37.0 $\pm$ 1.7  | 15.9 $\pm$ 3.0              |
|                            | 1.0                            |               | 138.9 $\pm$ 5.5                  | 40.2 $\pm$ 1.1  | 70.6 $\pm$ 2.0              |

<sup>a</sup> GABA =  $\gamma$ -aminobutyric acid. <sup>b</sup> Uptake measured in mouse whole brain minislices. <sup>c</sup> All values are mean  $\pm$  SEM for six samples except where indicated and for the control group where 18 samples were used. <sup>d</sup> Mean  $\pm$  SEM for five samples.

tests the compounds being evaluated were administered by intraperitoneal injection.

Based on the data obtained from the initial screening, additional tests were performed on the piperidinyl esters using male Sprague-Dawley mice<sup>4</sup>. To assess the activity of these compounds against convulsions induced by the putative  $\gamma$ -aminobutyric acid antagonist, bicuculline, the experimental animals received a subcutaneous injection of 3 mg/kg of bicuculline in a volume of 0.01 ml/g. The bicuculline solution was freshly prepared on the day of the experiment by dissolving the solid in 0.1 N HCl and adjusting to pH 5 with 0.1 N NaOH solution (20). The compounds evaluated against bicuculline-induced seizures were administered by intraperitoneal or subcutaneous injection prior to challenge with the convulsant. Diazepam, dissolved in a 40% propylene glycol–10% ethanol solution, was used as a reference compound in these studies.

The mice were divided into groups of four to eight animals, with Group 1 receiving injection of 0.9% NaCl solution (0.01 ml/g sc); Group 2, an injection of the diazepam solvent (0.01 ml/g ip); Group 3, 10 mg/kg ip of diazepam by injection; and Group 4, 150 mg/kg sc of the ester by injection. The incidence of clonic and tonic convulsions induced by bicuculline was determined at 0.5, 1.0, and 4.0 hr after administration of the test compound. A clonic convulsion was defined as a single episode of clonic spasms of at least a 5-sec duration. A tonic seizure was defined as a brief period of hindlimb flexion followed by a prolonged period of hindlimb extension.

In addition, the piperidinyl esters were reevaluated for their ability to protect against pentylenetetrazol-induced seizures. In the initial evaluation, the test compounds were administered by intraperitoneal injection. It was theorized that subcutaneous administration might increase anticonvulsant activity by preventing the peripheral hepatic hydrolysis of the esters that might occur with intraperitoneal dosing. Therefore, experimental animals received a subcutaneous injection of 85 mg/kg of pentylenetetrazol in a 0.01-ml/g volume. Sodium phenobarbital dissolved in saline was used as a reference compound. Groups of four to eight mice were used to determine the anticonvulsant activity, with Group 1 receiving a subcutaneous injection of 0.9% NaCl solution (0.01 ml/g); Group 2, 25 mg/kg sc of sodium phenobarbital by injection; and Group 3, 150 mg/kg sc of the ester by injection. The incidence of clonic and tonic convulsions induced by pentylenetetrazol was determined as described for bicuculline.

**Uptake of Amino Acids**—The uptakes of [<sup>3</sup>H] $\gamma$ -aminobutyric acid and tritiated L-proline were carried out according to described procedures (6, 21).

## REFERENCES

- (1) J. K. Saelens and F. J. Vinick, in "Annual Report in Medicinal Chemistry," vol. 13, F. H. Clarke, Ed., Academic, New York, N.Y., 1978, p. 31.
- (2) D. R. Curtis, in "GABA-Neurotransmitters, Pharmacological, Biochemical and Pharmacological Aspects," P. Krogsgaard-Larsen, J. Scheel-Kruger, and H. Kofod, Eds., Academic, New York, N.Y., 1979, p. 18.
- (3) B. S. Meldrum, *Int. Rev. Neurobiol.*, **17**, 1 (1975).
- (4) W. Löscher, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **315**, 119 (1980).
- (5) P. Krogsgaard-Larsen and G. A. R. Johnston, *J. Neurochem.*, **25**, 797 (1975).
- (6) J. D. Wood, D. Tsui, and J. W. Phillis, *Can. J. Physiol. Pharmacol.*, **57**, 581 (1979).
- (7) G. A. R. Johnston, P. Krogsgaard-Larsen, A. L. Stephanson, and B. Twitchin, *J. Neurochem.*, **26**, 1029 (1976).
- (8) D. Lodge, G. A. R. Johnston, D. R. Curtis, and S. J. Brand, *Brain Res.*, **136**, 513 (1977).
- (9) R. W. Horton, J. F. Collins, G. M. Anlezark, and B. S. Meldrum, *Eur. J. Pharmacol.*, **59**, 75 (1979).
- (10) H. H. Frey, C. Popp, and W. Löscher, *Neuropharmacology*, **18**, 581 (1979).
- (11) J. D. Wood, A. Schousboe, and P. Krogsgaard-Larsen, *ibid.*, **19**, 1149 (1980).
- (12) E. Falch, P. Krogsgaard-Larsen, and A. V. Christensen, *J. Med. Chem.*, **24**, 285 (1981).
- (13) P. Krogsgaard-Larsen and G. A. R. Johnston, *J. Neurochem.*, **30**, 1377 (1978).
- (14) P. Krogsgaard-Larsen, G. A. R. Johnston, P. Lodge, and D. R. Curtis, *Nature (London)*, **268**, 53 (1977).
- (15) J. March, in "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure," McGraw-Hill, New York, N.Y., 1968, p. 320.
- (16) L. J. Mathias, *Synthesis*, **1979**, 561.
- (17) J. A. Vida, in "Principles of Medicinal Chemistry," 2nd ed., W. O. Foye, Ed., Lea and Febiger, Philadelphia, 1981, p. 183.

<sup>4</sup> Obtained from Harlan Sprague-Dawley, Madison, Wis.

- (18) M. Freifelder, *J. Org. Chem.*, **28**, 1135 (1963).  
 (19) A. M. Crider, T. M. Kolczynski, and D. L. Miskell, *J. Pharm. Sci.*, **70**, 192 (1981).  
 (20) J. B. Patel, L. R. Nelson, and J. B. Malick, *Brain Res. Bull. Supp* **2**, 5, 639 (1980).  
 (21) L. L. Iversen and M. J. Neal, *J. Neurochem.*, **15**, 1141 (1968).

#### ACKNOWLEDGMENTS

This investigation was supported by a research grant from the Epilepsy

Foundation of America (A.M.C.). J. D. Wood gratefully acknowledges the grant support of the Medical Research Council of Canada. The authors thank Dr. W. E. Scott at Hoffmann-LaRoche for a generous supply of diazepam.

The arrangement of anticonvulsant testing through the Antiepileptic Drug Development Program administered by the National Institutes of Health by Mr. Gill D. Gladding is greatly appreciated.

The authors thank Catherine Forster, David Miskell, and Eugene Kurylo for their technical assistance, and the helpful suggestions by Dr. K. A. Bachmann are appreciated.

## Simultaneous Determination of Hydrocortisone and Benzyl Alcohol in Pharmaceutical Formulations by Reversed-Phase High-Pressure Liquid Chromatography

ALBERT REGO<sup>\*</sup> and BRETT NELSON

Received May 13, 1981, from the Analytical Development Group, Allergan Pharmaceuticals, Inc., Irvine, CA 92713.

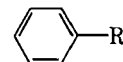
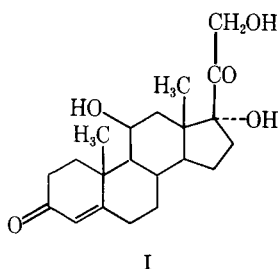
Accepted for publication January 12, 1982.

**Abstract** □ An accurate, reproducible, and specific reversed-phase high-pressure liquid chromatographic (HPLC) procedure that simultaneously determines hydrocortisone and benzyl alcohol in a variety of pharmaceutical formulations is presented. Cream, gel, ointment, and solution formulations containing varying hydrocortisone and benzyl alcohol concentrations can be analyzed with only minor modifications in sample preparation. To provide optimum accuracy and reproducibility, phenethyl alcohol is used as an internal standard. The specificity of the procedure allows for the quantitative determination of hydrocortisone and benzyl alcohol in the presence of their degradation products and without interference from the phenethyl alcohol. The determinations can be performed with an analysis time of 10–13 min/sample.

**Keyphrases** □ Hydrocortisone—simultaneous determination with benzyl alcohol, pharmaceutical formulations, reversed-phase high-pressure liquid chromatography □ Benzyl alcohol—simultaneous determination with hydrocortisone, pharmaceutical formulations, reversed-phase high-pressure liquid chromatography □ High-pressure liquid chromatography, reversed-phase—hydrocortisone, simultaneous determination with benzyl alcohol, pharmaceutical formulations

Hydrocortisone (I) has gained wide acceptance as a topical agent for the relief of inflammatory manifestations of corticosteroid-responsive dermatoses (1). Benzyl alcohol (II) is commonly used as an antimicrobial agent in a variety of topical formulations (2).

Various methods have been used for the determination of hydrocortisone, including TLC, polarography, colorimetry, GC, and high-pressure liquid chromatography (HPLC). TLC, though somewhat specific, lacks precision and short analysis time (1, 3–6). Polarographic determinations can be erroneous due to interferences, and they can



II: R = CH<sub>2</sub>OH  
 III: R = CH<sub>2</sub>CH<sub>2</sub>OH

be nonspecific in that a variety of similar steroids can elicit the same reduction potential for the same functional groups (7–10). Colorimetric methods include reaction with aldehyde-sulfuric acid (11), ammonium molybdate (12), and 4,5-dimethyl-*o*-phenylenediamine (13). These methods lack specificity, because degradation products may provide a colorimetric response not indicative of actual steroid concentration. Numerous GC methods are available (14–18); however, most steroids cannot be directly analyzed but must be initially derivatized. The complexity of steroids precludes the direct formation of a single derivative (19). The USP methods for hydrocortisone formulations were recently changed from a colorimetric reaction with tetrazolium blue (1, 20–23) to HPLC procedures (1). Recently, literature on hydrocortisone analysis *via* HPLC has been published (19, 24–29). While these methods may be specific, the sample preparation and analysis time can be prohibitive to a rapid assay. Benzyl alcohol can be determined by spectrophotometry and GC. Spectrophotometric methods have the inherent problems of nonspecificity and formulation base interferences. GC methods, including the USP compendial method, are specific and efficient (2). However, these methods may have interferences due to longer retained compounds from the formulation base<sup>1</sup>.

The described procedure allows for the simultaneous determination of hydrocortisone and benzyl alcohol in cream, gel, ointment, and solution formulations with a general sample preparation. The procedure incorporates phenethyl alcohol (III) as an internal standard to achieve optimum accuracy and reproducibility with an analysis

<sup>1</sup> Unpublished data.