decanted and the procedure was repeated. (This was to separate starting material which is soluble in ether.) The residue was evacuated over P_2O_5 to give a solid glass which was dissolved in EtOH and saturated with HCl. This was evaporated to a syrup which was dissolved in 6 ml of EtOH to give 11 ml of solution, which was seeded with material prepared previously by a similar process. After the mixture had been cooled overnight the product was collected (yield 4.2 g) and recrystallized from 10 ml of EtOH; yield 3.4 g (23%), mp 143–148°, λ_{max}^{Nujol} 3.95 and 5.83 μ. *Anal.* ($C_{12}H_{27}Cl_2N_3O_4$) C, H, Cl, N.

trans-**2-Dimethylaminocyclopentanol** (**XXII**).—Cyclopentane oxide (9.6 g, 0.114 mole) was added to a solution of 50 ml of H_2O previously saturated with anhydrous Me_2NH in an ice bath. The reaction mixture was stoppered and allowed to stand at room temperature for 5 days. The H_2O and excess Me_2NH were removed at reduced pressure on a water bath and the residue was distilled; yield 11.2 g (76%), bp 95–96° (10 mm), $n^{25}D$ 1.4727 (lit.[21] bp 94–95° (11 mm), $n^{25}D$ 1.4710). *Anal.* ($C_7H_{15}NO$) N.

(±)-*erythro*-**1-Phenyl-2-dimethylaminopropanol** (**XXIII**).—(±)-*erythro*-1-Phenyl-2-aminopropanol hydrochloride (mp 194–197°) (18.8 g, 0.10 mole), 12 ml of H_2O, 85 ml of 97% HCOOH, and 85 ml of 37% formaldehyde were mixed and heated on a steam bath for 19 hr. The reaction mixture was evaporated to dryness at reduced pressure, dissolved in 75 ml of H_2O, and again evaporated. This latter procedure was repeated twice more and the resulting crystalline residue was recrystallized from 50 ml of 95% EtOH; yield 4.7 g, mp 207–209°. Additional materials, 4.95 g, mp 204–206.5°, and 6.25 g, mp 204–207°, were obtained from the filtrate (74% total yield). *Anal.* ($C_{11}H_{18}ClNO$) C, H, Cl, N. This hydrochloride (13.0 g, 0.06 mole) was dissolved in 25 ml of H_2O and 15 ml of 10 *N* NaOH was added. The mixture was extracted with three 100-ml portions of ether which were combined and dried ($MgSO_4$). Evaporation of the ether gave a white crystalline solid; yield 10.6 g, mp 60.5–65°. A small portion of this compound was recrystallized from petroleum ether; mp 65–67.5°.

Bis(2-diethylaminoethyl) 4,4′-Ethylenedi(1-piperazinecarboxylate) (**96**).—A solvent was included in this experiment to prevent premature precipitation of a monoalkylation product. A solution of 9.37 g (0.05 mole) of $BrCH_2CH_2Br$ and 22.93 g (0.1 mole) of 2-diethylaminoethyl piperazine-1-carboxylate (**2**) in 100 ml of EtOH was heated under reflux for 15 hr. A little solid had separated. The mixture was evaporated to dryness and the residue dissolved in 20 ml of hot H_2O. This solution was chilled during the portionwise addition with shaking of 11.22 g (0.2 mole) of KOH. The resulting slush was extracted by decantation with 100 ml, then three 20-ml portions of ether. The ethereal extracts were dried (K_2CO_3), filtered, then evaporated. The residual oil was distilled *in vacuo* without a fractionating column, using a Claisen distillation head leading directly to a vacuum adapter connected to the receiver. The adapter alone provided ample condensing surface. An oil bath heated with a hot plate gave only enough heat for removal of a forerun. Much more effective heating was provided by an "air bath chimney" made from two layers of aluminum foil formed into a cylinder and suspended from a metal ring. A wire gauze with a center circle of asbestos hung from three wires which held it near the bottom of the chimney. The gauze was heated with a wide, soft flame from a Mekker burner. The still pot was not clamped in place. Instead, the pot, Claisen head, adapter, and receiver (all with 24/40 ⅋ glass joints) were wired together. Thus all of the pot and part of the Claisen head could be heated in the chimney. The whole assembly balanced nicely, the side arm of the Claisen head resting on the foil-covered iron ring. An ebullator was unnecessary; the pot charge was readily kept swirling by gently rocking the whole assembly of glassware. There was no sign of charring or decomposition in the pot. An intermediate cut amounted to 0.62 g, bp ~246° (0.09 mm), $n^{25.2}D$ 1.4946. Collection of the product was begun when the distillate no longer darkened as it ran down the adapter. The product was 16.2 g (67%) of a very viscous, light yellow oil which later crystallized completely; bp 250–255° (0.1 mm), $n^{25.2}D$ 1.4942, mp 30–32°. *Anal.* ($C_{24}H_{48}N_6O_4$) C, H, N.

2-Diethylaminoethyl 2-Methoxyethyl Carbonate (**100**).—A procedure used for the preparation of *n*-butyl chloroformate[22] was adapted for the synthesis of 2-methoxyethyl chloroformate; 96% yield, bp 70° (30 mm), $n^{26.6}D$ 1.4161 (lit.[23] bp 59° (13 mm), $n^{25}D$ 1.4163). A solution of 17.60 g of 2-diethylaminoethanol in 75 ml of CH_2Cl_2 was gradually added to a cold solution of 20.80 g of 2-methoxyethyl chloroformate in 150 ml of CH_2Cl_2. After 5 days at about 26° the mixture was worked up as described in procedure C, except that the basification was with 20.0 g of anhydrous K_2CO_3. Two successive fractionations gave 14.78 g (45%) of product, bp 72° (0.06 mm), $n^{26.7}D$ 1.4319, λ_{max}^{neat} 5.73 and 7.85 μ. *Anal.* ($C_{10}H_{21}NO_4$) C, H, N.

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Structural Aspects of Picrotoxinin Action

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Received January 15, 1968

A number of compounds related to picrotoxinin have been tested for ability to mimic the action of that compound. Active compounds have a free bridgehead hydroxyl group and a lactone ring connecting carbons 3 and 5 of the picrotoxane (**I**) skeleton. The axial isopropenyl substituent group of picrotoxinin appears to have a role in determining potency.

Picrotoxin, recently reviewed by one of us,[1] is an ancient[2–4] drug component of *Anamirta paniculata* and *cocculus*. It is an efficient analeptic,[5] but seldom used. Despite its therapeutic obsolescence picrotoxin is of much theoretical interest because of its site and mode of action in the central nervous system. It appears to competitively depress presynaptic inhibition in the vertebrate spinal cord and not to effect postsynaptic inhibitory processes.[6] The anatomical specificity in action prompted our interest. The generalization that only its picrotoxinin (II) component was active and that picrotin (III) was inactive was also of interest since their only difference is hydration of the isopropenyl group. In connecting this information it was thought that presynaptic inhibitory receptors should show high structure discrimination and that structure–activity re-

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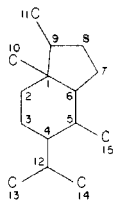

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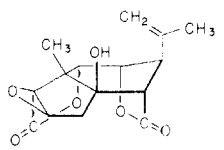
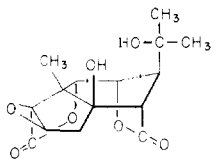


II



III

lationship studies using picrotoxinin as a model could assist in gaining incipient cognizance of receptor chemical topography. To that end we have made a preliminary investigation of structure–activity relationships in a series of compounds derived from picrotoxinin and other related naturally occurring lactones. This study was made on whole animals and the criteria used for activity comparisons were the CD_{50} (median convulsive dose) and the LD_{50}. These parameters by no means establish that any effect is due to activity at presynaptic inhibitory synapses. However, assuming that uptake and distribution factors are approximately equal the data are sufficient to screen inactive compounds from ones of medium or high activity. On this basis the measurements can be used to study the over-all effects of structure on biological activity and to select compounds for microelectrode studies which do reveal the site of action and permit a reasonable evaluation of receptor affinity and intrinsic activity.

Experimental Section

Microanalysis for carbon and hydrogen were by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elemental functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values. All melting points listed were taken with the Fisher-Johns apparatus and are corrected. Ir spectra of all compounds were determined using KCl pellets and a Perkin-Elmer Model 237 grating-type spectrometer. Pmr spectra were determined in $CDCl_3$ using a Varian Model A-60-A spectrometer.

Determination of Median Convulsive and Median Acute Toxic Doses.—The CD_{50} and LD_{50} values, Table I, were determined using separate groups of virgin female Swiss–Webster albino mice 45–59 days old and weighing approximately 20 g each. Each value was determined approximately by using single mice at different dose levels. When an approximate value was found, more precise data were developed by using four mice at each of four doses and plotting a dose–response curve. The determinations were all made in an environment of minimum noise and vibration with controlled temperature and light. The individual mice were isolated for the determinations. The compounds were ground with 0.25% aqueous methylcellulose for 5 min in a tissue grinder. All injections were made intraperitoneally and on a mg/kg of mouse basis. In no case did the injected volume total more than 1.0 ml. In those cases where no effect was noted the number listed is the highest dose tested.

Details of synthesis not appearing below are available on request to the senior author.

Picrotoxinin Benzoate.—Picrotoxinin (2 g) was refluxed for 2 hr in 9 ml of redistilled benzoyl chloride. The mixture was cooled and poured into 200 ml of 2 *N* Na_2CO_3 and stirred overnight. The precipitate was filtered, decolorized, and crystallized from EtOH to give 2.2 g of stout white needles, mp 210–212°; mixture melting point with picrotoxinin was 175–200°. *Anal.* ($C_{22}H_{20}O_7$) C, H. The ir spectrum showed maxima at 1800 (broad), 1715 (broad), 1650, 1600, 1585, and 1455 cm^{-1}. The nmr spectrum showed two nearly superimposed 3 H signals at τ 8.51 and 8.52 and 5 aromatic protons in the region 1.80–2.70.

Tutin Diacetate.—Tutin (0.1 g) was refluxed for 24 hr in 5 ml of Ac_2O containing 40 mg of anhydrous NaOAc. The reaction mixture was cooled and poured into ice water. The precipitate was filtered, decolorized, and crystallized from H_2O to give 0.06 g of product, mp 179–184°. Recrystallization from EtOH–H_2O gave product with mp 197–201°. *Anal.* ($C_{19}H_{22}O_8$) C, H. The ir spectrum showed maxima at 1790 (strained lactone), 1745, 1730 (esters), and 1650 cm^{-1} (C=CH_2). The nmr spectrum was very similar to that of tutin.

Ethyl Bromopicrotoxinate.—Bromopicrotoxinin (1 g) was dissolved in 30 ml of dioxane and combined with 2 g of Zn–Cu couple, 2 ml of CH_2I_2, and 1 ml of absolute EtOH. The reaction was refluxed for 3 days with an additional 1 ml of EtOH being added at the end of days 2 and 3. The mixture was then neutralized with 10% aqueous Na_2CO_3. The filtered solution was evaporated to give a residue, which was crystallized from H_2O to give 150 mg of product, mp 177°. *Anal.* ($C_{18}H_{22}BrO_6$) C, H.

TABLE I

CONVULSANT AND TOXIC DOSES OF PICROTOXININ-RELATED COMPOUNDS[a]

No.	Compd	CD_{50}, mg/kg	LD_{50}, mg/kg
1	Picrotoxinin[b]	1.5	3.0
2	Tutin	1.5	3.0
3	Coriamyrtin	1.6	3.0
4	α-Dihydropicrotoxinin[b,c]	8.0	14
5	Tutin monoacetate[d,e]	43	80
6	Picrotin[e]	80	135
7	α-Picrotoxinone[f,g]	100	120
8	Ethyl bromopicrotoxate	160	185
9	α-Dihydropicrotoxinin acetate[h]	180	220
10	Methyl α-picrotoxinate[i]	300	400
11	Methyl β-picrotoxinate[i,j]	300	400
12	Picrotin monoacetate[k,l]	400	800
13	Picrotoxinin acetate[h]	Inactive at 272 mg/kg	
14	α-Picrotoxinic acid[i]	Inactive at 300 mg/kg	
15	β-Picrotoxinic acid[i]	Inactive at 300 mg/kg	
16	Methyl picrotoxate[m]	Inactive at 300 mg/kg	
17	Methyl dihydropicrotoxate[m]	Inactive at 300 mg/kg	
18	Neopicrotoxinin acetate[h]	Inactive at 360 mg/kg	
19	Bromopicrotoxinin[n]	Inactive at 400 mg/kg	
20	Neopicrotoxinin[e,o]	Inactive at 400 mg/kg	
21	Neopicrotoxinin benzoate[e,q]	Inactive at 400 mg/kg	
22	Picrotoxinin benzoate	Inactive at 400 mg/kg	
23	Tutin diacetate	Inactive at 400 mg/kg	
24	α-Picrotoxinone acetate[f]	Inactive at 400 mg/kg	
25	Bromoneopicrotoxinin[n]	Inactive at 400 mg/kg	
26	Anhydropicrotin[p]	Inactive at 400 mg/kg	
27	β-Picrotoxinone[f]	Inactive at 800 mg/kg	

[a] Compounds **13–27** are apparently inactive as convulsants. The maximum dose used is cited. [b] C. H. Jarboe and L. A. Porter, *J. Chromatog.*, **19**, 427 (1965). [c] Mp 259–260°, lit.[d] mp 250°. [d] S. N. Slater, *J. Chem. Soc.*, 50, 143 (1943). [e] Mp 181–183°, lit.[d] mp 177°. [f] P. I. Burkhill and J. S. E. Holker, *J. Chem. Soc.*, 4011 (1960). [g] Mp 205–207°, lit.[f] mp 179–184°. [h] D. E. Hathway, *J. Chem. Soc.*, 4953 (1957). [i] P. Horrmann, *Ber.*, **46**, 2793 (1913). [j] Mp 178–179°, lit.[i] mp 182°. [k] P. Horrmann, *Ber.*, **43**, 1903 (1910). [l] Mp 255–257°, lit.[k] mp 237°. [m] J. C. Beunstead, R. Cee, R. B. Johns, M. Martin-Smith, and S. N. Slater, *J. Chem. Soc.*, 2292 (1952). [n] S. N. Slater, *ibid.*, 806 (1949). [o] Mp 223–225°, lit.[n] mp 204, 212, 220°. [p] J. S. E. Holker, A. Robertson, J. H. Taylor, K. V. Holker, and W. B. N. Williamson, *J. Chem. Soc.*, 2987 (1958). [q] Mp 255–257°, lit.[p] mp 249–250°.

Discussion

Table I summarizes the results of this study. The quantitative differences in the CD_{50} and LD_{50} values are so great that it is possible to divide the compounds into four activity classes. Compounds **1–4** constitute the first or high activity class containing the natural products and α-dihydropicrotoxinin. Tutin monoacetate comprises the second or intermediate activity class, picrotin and α-picrotoxinone the third low activity class, and compounds **8–27**, the fourth, are inactive. From

these data it is clear that a structural dependence exists in the convulsant activity of these lactones and even though each compound contains the picrotoxane skeleton[1] there is sufficient structural diversity to comment on their biochemorphology. Each high activity compound has a lactone function connecting C-3 and -5 of the skeleton. The carbonyl system is *cis* to the fused ring structure and, in combination with the bridgehead hydroxyl at C-6, appears to comprise the absolute structural requirements for activity. In almost every case where either the hydroxyl is protected, *e.g.*, picrotoxinin acetate, or where the lactone system joining C-3 and -5 is absent, *e.g.*, methyl α-picrotoxinate, there is no activity. There is one exception to this generalization and that is α-dihydropicrotoxinin acetate, a compound hydrolyzed to α-dihydropicrotoxinin with great ease. In our view the activity of this compound is due to its hydrolysis product.

From the structures and high activity of picrotoxinin, tutin, and coriamyrtin it appears that oxirane ring location and even perhaps its presence have no role in the biological effect. There also appears to be no requirement for the lactone ring connecting C-2 and -13 in picrotoxinin since the other naturally occurring compounds, all possessing equal activity, do not contain this group.

The substitution pattern at C-4 appears to have a role in determining whether compounds with the requisite lactone and hydroxyl groups are highly active. All the compounds with CD_{50} values at about 1.5 mg/kg have an isopropenyl group *trans* to the lactone and *cis* to the bridgehead hydroxyl. Interaction between the π electrons of the double bond and the proton of the hydroxyl has been well established.[7,8] In α-dihydropicrotoxinin this interaction is precluded and, while that compound is highly active, it has potency about one-fifth that of the parent. Picrotin, containing a hydroxyl group at C-12 instead of hydrogen as in dihydropicrotoxinin, has low activity, a greater steric requirement, and no π-electron interaction with its bridgehead hydroxyl. The indications are that hydrogen bonding of the bridgehead hydroxyl and the π electrons of the isopropenyl group are involved in determining receptor affinity and that C-4 is bulk sensitive to substituents which inhibit access to the lactone system. This is seen in the inactivity of neopicrotoxinin. This compound should be active if the sole activity requirements were the lactone and hydroxyl groups. However, its isopropylidine group is not axial but in the plane of picrotoxane carbons 3, 4, and 5. This yields a greater steric requirement, interrupts the π-electron–hydroxyl group interaction of picrotoxinin, and deshields the hydroxyl.

Additionally, tutin has a free hydroxyl substituent at C-4 of the picrotoxane skeleton. This hydroxyl is *cis* to the lactone and in such close proximity that interconversion of the lactone from a five- to a six-membered ring is possible. On esterification the free OH is converted to the much larger acetoxy group. This could, and apparently does, partially impede approach of the lactone to the concerned receptor since the activity of tutin monoacetate is intermediate.

Although these compounds give an insight into lactone analeptic biochemorphology, they do not permit assessment of bridgehead methyl group importance or that of the fused-ring system. To that end appropriate lactones of various cyclohexanecarboxylic acids are under investigation. Preliminary experiments indicate that simple compounds containing the lactone and hydroxyl groups in the axial–axial arrangement are active.

Acknowledgments.—This work was supported by research Grant MH-08348 and Training Grant 2T 1 GM 461 from the National Institutes of Health, U. S. Public Health Service. The authors are indebted to Mr. C. James Rosene for proton magnetic resonance spectra of the compounds; to Dr. Jiancarlo Jommi, Organic Chemistry Institute, University of Milan, Milan, Italy, for information on the biological activity of hyenancin, isohyenancin, and capenecin; to Dr. Takuo Okuda, Kyoto University, Kyoto, Japan, for a sample of coriamyrtin; and to Dr. W. E. Harvey, Victoria University of Wellington, Wellington, New Zealand, for a sample of tutin.

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Some 4(1H)-Quinolylidene and 1,8-Naphthyrid-4(1H)-ylidene Compounds

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Received January 25, 1968

The synthesis and antiinflammatory activity of 4-quinolylacetamide, 7-methyl-1,8-naphthyrid-4-ylacetamide, some novel 4(1H)-quinolylideneacetamides and 1,8-naphthyrid-4(1H)-ylideneacetamides, and also three 2,3,4,6-tetrahydropyrido[3,4-*c*]quinoline-2,4-diones are reported. The most active compound of the series was found to be 1-ethyl-7-methyl-1,8-naphthyrid-4(1H)-ylideneacetamide.

Since the search for new antiinflammatory compounds began in these laboratories, a large number of heterocyclic carboxylic acids and many of their derivatives have been synthesized and screened for biological activity. Among these were the substituted anilinopyridinecarboxylic acids reported by Evans, *et al.*[1]

In connection with this program it recently became of interest to prepare a number of substituted acetamides and related compounds.

Chemistry.—Borror and Haeberer[2] reported the reaction between 2-chloroquinoline and sodium ethyl cyanoacetate to give ethyl cyano-2(1H)-quinolylidene-

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