Journal of Medicinal Chemistry, 1975, Vol. 18, No. 2 189

# Chemistry and Antiinflammatory Activities of Prodolic Acid and Related 1,3,4,9-Tetrahydropyrano[3,4-*b*]indole-1-alkanoic Acids. 1†

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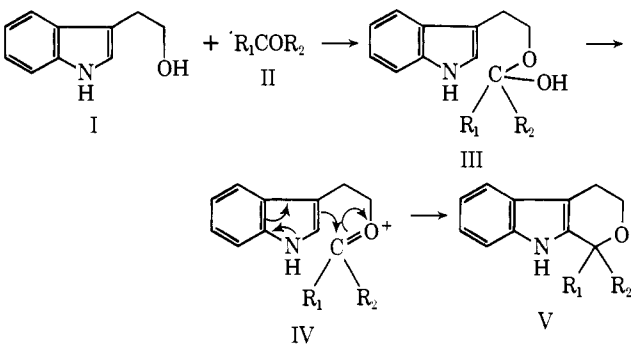
The synthesis and antiinflammatory activities of a series of 23 novel 1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-alkanoic acids are described and some relationships between structure and activity are discussed. One of these compounds, 1,3,4,9-tetrahydro-1-propylpyrano[3,4-*b*]indole-1-acetic acid (prodolic acid, USAN), has been selected for further studies.

A versatile method for the synthesis of a variety of novel pyranofused heterocyclic systems has recently been developed in our laboratories.[1] This report describes an application of this synthetic method for the preparation of a series of novel 1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-alkanoic acids and their derivatives. Some of these compounds were found to be potent antiinflammatory agents. Results of screening and structure-activity relationships are discussed.

**Chemistry.** The synthetic route used[1] involved the acid-catalyzed (*p*-TSA or $P_2O_5$) intramolecular alkylation at the 2 position of the indole nucleus by a hemiketal III, or a derived oxonium ion intermediate IV, formed *in situ* from the reaction of an indole-3-ethanol (tryptophol) I and a keto ester $R_1COR_2$ II (see Table I for definitions of $R_1$ and $R_2$). The 1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-alkanoic acid esters obtained from this reaction were hydrolyzed directly to the corresponding 1-alkanoic acids V



which in a few instances were transformed to amide or ester derivatives. The pyrano[3,4-*b*]indole ring system has been synthesized previously[2-4] but only by methods that generate higher oxidation states than the 1,3,4,9-tetrahydro derivatives of formula V and which are unsuitable for the introduction of functional substituents at position 1. Chemical data on 23 compounds of type V, prepared for antiinflammatory testing, are collected in Table I. Details concerning their syntheses are described in the Experimental Section and in the footnotes to Table I.

**Pharmacology and Structure-Activity Relationships.** The compounds in Table I were tested for antiinflammatory activity in groups of six rats with established adjuvant arthritis ("therapeutic test") selected for uniformity of arthritic lesions, as described previously.[5] Oral treatment with compounds was started 14 days after adjuvant (*Mycobacterium butyricum* in mineral oil) injection in the foot pad of the left hindpaw and continued until day 22 (9 po administrations). A decrease of the volume of the injected paw, of 0.5 ml (approximately 50% of the maximum possible decrease) or more, as a result of drug treatment was considered to be a "therapeutic effect." Smaller changes were considered to be negative. Paw volume was measured by mercury displacement. From the number of rats showing a "therapeutic effect," the therapeutic $ED_{50}$ was calculated by probit analysis.[6] Compounds which failed to decrease the injected paw size by 0.5 ml in any of the rats at the arbitrarily closed screening dose (25–100 mg/kg) were considered inactive. Phenylbutazone was tested for comparison purposes.

The first compound examined in this series, the 1-methylacetic acid (**1**), had weak antiinflammatory activity ($ED_{50}$ ~200 mg/kg). Activity was not increased by a variety of moleular manipulations, such as lengthening the acetic acid side chain (**2**, **3**), transformation to an ester or amide (**4**, **6**) or, alkylation of the indolic nitrogen (**7**, **8**). Similarly, the isomeric $\alpha$-methylacetic acids **9** and **10** were devoid of activity. Variation in the length of the 1-alkyl chain from methyl to *n*-butyl revealed that the 1-ethyl (**11**) and 1-*n*-propyl (**12**)‡ analogs were over 20 times more active than the 1-methyl analog **1** and comparable to phenylbutazone **24** in activity. Branching of the 1-alkyl chains (**17**, **24**) or introduction of aryl residues at the 1 position gave compounds with little or no activity. The spirocyclopentane derivative **18**, which corresponds to joining the *n*-propyl chain of **12** to the carbon $\alpha$ to the carboxyl group, was inactive.

The effects of methyl substitution at positions 3 and 4 of the 1-ethyl derivative **11** were studied. Thus, the two isomeric 3-methyl-1-ethyl analogs **19** and **20** were synthesized, as well as the isomeric pair of 4-methyl-1-ethyl analogs **21** and **22**. It was not possible to assign the stereochemistry of these isomeric pairs by nmr spectroscopy. The 3-methyl isomer **20** retained some of the activity of the parent structure **11**, but its epimer **19** was inactive. The 4-methyl isomers exhibited a striking separation of antiinflammatory activities with **22** being at least eight times more active than its epimer **21** and twice as active as phenylbutazone **24**. The 4,4-dimethyl analog **23** was inactive.

The large difference in activity observed between the epimeric 4-methyl derivatives **21** and **22** suggests that the configuration at position 4 plays a critical role in determining the activity of these compounds.

Also, the weak activity of the 1-methyl analog **1**, compared to the high activity of the 1-ethyl and 1-*n*-propyl

† Presented in part at the 14th Medicinal Chemistry Symposium of the American Chemical Society, Durham, N.H., June 1974.

‡ Prodolic acid (USAN); also known by Ayerst code number AY-23,289. This compound has been selected for further studies and its detailed pharmacological profile is the subject of a forthcoming publication.[7]

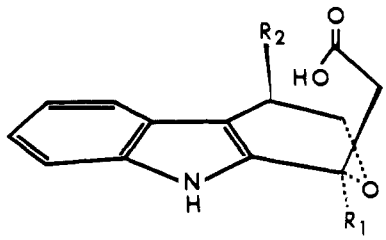


Figure 1.

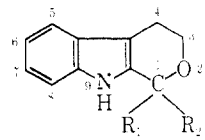
analogs 11 and 12, suggests that the size of the 1-alkyl substituent plays a critical role in determining the potencies of 11 and 12.

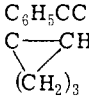
The dependence of antiinflammatory activity on these two apparently independent parameters is readily rationalized by the hypothesis that the acetic acid chain must be situated in a plane above that of the pyranoindole nucleus (see Figure 1) in order to interact with an "antiinflammatory receptor." Thus, when the 1-alkyl substituent is an ethyl or *n*-propyl group the acetic acid chain would be expected to be pseudo-axial with respect to the dihydropyran ring, and when it is a small group such as a methyl, it would be expected to be pseudo-equatorial. The additional postulate that the pseudo-axial acetic acid chain must assume a conformation at the receptor in which the bond between the methylene and carboxyl groups is directed toward $C_4$ accounts for the difference in activities between the 4-methyl epimers 21 and 22. Thus, a 4-methyl group located cis to the acetic acid group would prevent the carboxyl group from assuming its mandatory coordinates while a *trans*-4-methyl group would not exert such an effect.

On the basis of these considerations we propose that prodolic acid and the related active analogs described above exert their activity by interaction with a receptor which accommodates the tricyclic pyranoindole nucleus and a carboxyl group (or a carboxylate anion) which is located at the center of the dihydropyran ring and in a plane which is approximately 2.5 Å above the plane of the indole nucleus.

While these considerations provide a rationalization for the observations cited above, they fail to explain the lack of activity of analogs bearing alkyl groups at position 1

Table I. Chemical and Antiinflammatory Data on Tetrahydropyrano[3,4-*b*]indole-1-alkanoic Acids[a]



| No. | $CR_1R_2$ | Other substituents | Mp, °C | Recrystn solvent[b] | Yield, % | Formula[c] | "Therapeutic test" in arthritic rats, $ED_{50}$ (mg/kg ± S.E.) |
|---|---|---|---|---|---|---|---|
| 1 | $MeCCH_2COOH$[d] | | 150–152 | A, B | 46 | $C_{14}H_{15}NO_3$ | ~200 |
| 2 | $MeC(CH_2)_2COOH$[e] | | 104–110 | B, C | 98 | $C_{15}H_{17}NO_3$ | Inactive[f] |
| 3 | $MeC(CH_2)_3COOH$ | | 132–135 | A, D | 44 | $C_{16}H_{19}NO_3$ | Inactive[f] |
| 4 | $MeCCH_2COOMe$[g] | | 87–90 | A, D | 90 | $C_{15}H_{17}NO_3$ | Inactive[f] |
| 5 | $MeCCH_2CONHMe$[e] | | 138–140 | B, C | 76 | $C_{15}H_{18}N_2O_2$ | Inactive[f] |
| 6 | $MeCCH_2CONMe_2$[e] | | 149–151 | C | 65 | $C_{16}H_{20}N_2O_2$ | Inactive[f] |
| 7 | $MeCCH_2COOH$[e] | 9-Me | 105–108 | A | 40 | $C_{15}H_{17}NO_3$ | Inactive[f] |
| 8 | $MeCCH_2COOH$[e] | 9-Allyl | 103–105 | A, D | 42 | $C_{17}H_{19}NO_3$ | Inactive[f] |
| 9 | $MeCCH(Me)COOH$[h] | | 154–156 | B, C | 13 | $C_{15}H_{17}NO_3$ | Inactive[f] |
| 10 | $MeCCH(Me)COOH$[h] | | 163–165 | B, C | 12 | $C_{15}H_{17}NO_3$ | Inactive[f] |
| 11 | $EtCCH_2COOH$ | | 137–140 | A, B | 62 | $C_{15}H_{17}NO_3$ | 6.8 ± 1.3 |
| 12 | *n*-$PrCCH_2COOH$ | | 152–153 | E | 52 | $C_{16}H_{19}NO_3$ | 9.0 ± 2.8 |
| 13 | 2-$PrCCH_2COOH$ | | 150–152 | A, B | 53 | $C_{16}H_{19}NO_3$ | Inactive[f] |
| 14 | *n*-$BuCCH_2COOH$ | | 124–127 | A, B | 31 | $C_{17}H_{21}NO_3$ | Inactive[f] |
| 15 | *t*-$BuCCH_2COOH$ | | 210–212 | A | 25 | $C_{17}H_{21}NO_3$ | 75 ± 12 |
| 16 | 2-$C_4H_3SCCH_2COOH$[i] | | 127–130 | A, B | 36 | $C_{17}H_{15}NO_3S$ | Inactive[f] |
| 17 | $C_6H_5CCH_2COOH$ | | 148–150 | A, B | 42 | $C_{19}H_{17}NO_3$ | Inactive[f] |
| 18 |  | | 168–170 | F | 37 | $C_{16}H_{17}NO_3$ | Inactive[j] |
| 19 | $EtCCH_2COOH$ | 3-Me[h] | 145–146 | A, B | 8 | $C_{16}H_{19}NO_3$ | Inactive[j] |
| 20 | $EtCCH_2COOH$ | 3-Me[h] | 147–148 | C, D | 8 | $C_{16}H_{19}NO_3$ | >25[k] |
| 21 | $EtCCH_2COOH$ | 4-Me[h] | 132–133 | A | 17 | $C_{16}H_{19}NO_3$ | >25[k] |
| 22 | $EtCCH_2COOH$ | 4-Me[h] | 153–154 | A, B | 28 | $C_{16}H_{19}NO_3$ | 3.3 ± 0.8 |
| 23 | *n*-$PrCCH_2COOH$ | 4,4-$Me_2$ | 184–185 | G | 24 | $C_{18}H_{23}NO_3$ | Inactive[j] |
| 24 | Phenylbutazone | | | | | | 5.4 ± 1.2 |

[a] The indole starting material for the preparation of these compounds was tryptophol except for 19 and 20 (α-methyltryptophol[8]), 21 and 22 (β-methyltryptophol[9]), and 23 (β,β-dimethyltryptophol[10]). The keto ester components were the ethyl esters corresponding to the formula $R_1COR_2$ (see column 2). These keto esters were all commercially available except for ethyl δ-ketohexanoate which was prepared from the corresponding acid. [b] A = benzene; B = petroleum ether, bp 60–90°; C = ethyl acetate; D = hexane; E = toluene; F = ether; G = acetonitrile. [c] All compounds were analyzed for C, H, and N. The results were within ±0.4% of the calculated values. [d] See Experimental Section for synthesis. Compounds 3, 11–18, and 23 were prepared by similar methods. Yields in column 6 refer to overall yields of crystalline acids based on the tryptophol used. [e] See Experimental Section. [f] Inactive at a dose of 100 mg/kg (highest dose tested). [g] Prepared from 1 by esterification with MeOH–HCl. [h] The synthesis, separation, and nmr spectra of the individual isomers of this pair are described in the Experimental Section. [i] $C_4H_3S$ = thienyl. [j] Inactive at a dose of 25 mg/kg (highest dose tested). [k] The compound reduced the paw size by 0.5 ml in some rats but the $ED_{50}$ was greater than 25 mg/kg.

which are larger or bulkier than *n*-propyl. The inactivity of these analogs (**13–15**) may be due to interference with a ligand-receptor interaction involving the indolic NH or to a supraoptimal effect on the local lipophilicity of the molecule.

## Experimental Section

All compounds had nmr and ir spectra consistent with their respective structures. Melting points were taken on a Thomas-Hoover apparatus and need no correction. Nmr spectra were determined using a Varian A-60A spectrometer and the chemical shifts ($\delta$) are reported as parts per million downfield from TMS.

**1,3,4,9-Tetrahydro-1-methylpyrano[3,4-*b*]indole-1-acetic Acid (1).** Ethyl acetoacetate (23.4 g, 0.18 mol) was added to a solution of tryptophol (10.0 g, 0.06 mol) in $C_6H_6$ (200 ml). *p*-TSA (1.9 g) was added and the mixture refluxed for 3 hr. The $C_6H_6$ solution was washed with 5% aqueous $NaHCO_3$ and worked up in the conventional manner to give an oil which was eluted from a silica gel column with benzene–ether (20:1) to afford the ethyl ester of the product still containing some ethyl acetoacetate. The oily mixture (19 g) was dissolved in MeOH (230 ml) and to this was added KOH (10.0 g) and $H_2O$ (30 ml). After stirring at 22° for 16 hr, a conventional work-up procedure afforded the product (9.0 g) as an oil. Crystallization from $C_6H_6$–petroleum ether gave the product (6.8 g), mp 150–152°.

**1,3,4,9-Tetrahydro-1-methylpyrano[3,4-*b*]indole-1-propionic Acid (2).** A mixture consisting of tryptophol (7.2 g, 0.04 mol), ethyl levulinate (6.4 g, 0.04 mol), $C_6H_6$ (200 ml), $P_2O_5$ (17 g), and Celite (3 g) was stirred at 22° for 17 hr. The Celite was removed by filtration and the filtrate was washed with 5% aqueous $NaHCO_3$ and then with $H_2O$. After drying and evaporation, the ethyl ester of the product was obtained. It was purified by eluting from a silica gel column with hexane–$Me_2CO$ (4:1). The ester was hydrolyzed with KOH in MeOH to give the product (4.7 g), mp 104–110° (EtOAc–petroleum ether).

**1,3,4,9-Tetrahydro-1-methylpyrano[3,4-*b*]indole-*N*-methylacetamide and -*N,N*-dimethylacetamide (5 and 6).** To compound **1** (20 g, 0.082 mol) in THF (320 ml) at −5° was added with stirring $Et_3N$ (24.8 g, 0.246 mol), followed by ClCOOEt (22.14 g, 0.205 mol). The mixture was kept at 0° for 2 hr; then it was added to a solution of 30% aqueous $MeNH_2$ (390 ml). After 30 min at 22°, the THF was removed *in vacuo* and the residue was partitioned between $CHCl_3$ and $H_2O$. A conventional work-up of the $CHCl_3$ phase gave an oil (17.5 g) which was filtered through a column of silica gel with $C_6H_6$–$Me_2CO$ (7:3). The eluates afforded pure **5** (16.0 g), mp 138–140°.

The use of $Me_2NH$, instead of $MeNH_2$, in the above procedure afforded compound **6**, mp 149–151°.

**9-Methyl- and 9-Allyl-1,3,4,9-tetrahydro-1-methylpyrano[3,4-*b*]indole-1-acetic Acids (7 and 8).** Compound **1** (10.0 g, 0.04 mol) in THF (150 ml) was added dropwise to NaH (4.4 g of a 55% dispersion in mineral oil) in THF (200 ml) and the mixture was heated at 50° for 2 hr. MeI (14.2 g, 0.10 mol) was added during 15 min and the mixture was heated for 2 hr at 50°. A conventional work-up procedure afforded the product (12.0 g), mp 105–108°.

The use of allyl bromide, instead of MeI in the above procedure, gave compound **8**, mp 103–105°.

**1,3,4,9-Tetrahydro-$\alpha$,1-dimethylpyrano[3,4-*b*]indole-1-acetic Acids (9 and 10).** A mixture of tryptophol (20 g, 0.125 mol), ethyl 2-methylacetoacetate (20.16 g, 0.140 mol), and *p*-TSA (2.0 g) was refluxed in $C_6H_6$ for 3 hr. The $C_6H_6$ solution was washed with 5% aqueous $NaHCO_3$, dried, and evaporated to afford an oil (40 g) which was a mixture of two compounds as shown by tlc. The mixture was chromatographed on silica gel. Elution with $Me_2CO$–hexane (1:4) gave the ethyl ester of **9** (26.0 g) which was hydrolyzed by heating for 3 hr in a mixture of MeOH (200 ml), $H_2O$ (20 ml), and KOH (26 g). The usual work-up procedure afforded **9**: mp 154–156°; nmr ($CDCl_3$) $\delta$ 1.07 (3, d, $J$ = 7 Hz, $CHCH_3$), 1.67 (3, s, $O(C)C(C)CH_3$), 2.7–3.25 (2, m, $OCH_2CH_2$), 3.1 (1, q, CHCO), 4.12 (2, m, $OCH_2$), 7.30 (4, m, aromatic protons), 9.04 (1, s, NH), 10.60 (1, s, COOH).

Further elution with $Me_2CO$–hexane (1:4) gave the ethyl ester of **10** (7.0 g), mp 147–149°. It was hydrolyzed by heating at reflux with LiI (10.2 g) in 2,6-lutidine (240 ml) for 10 hr. The mixture was partitioned between $CHCl_3$ and 2 *N* HCl. The $CHCl_3$ phase was washed with 2 *N* HCl and then $H_2O$, dried, and evaporated to afford an oil (3.3 g). This was purified by eluting from a silica gel column with $C_6H_6$–$Me_2CO$ (20:1) to afford **10** (1.8 g): mp 163–165°; nmr ($CDCl_3$) $\delta$ 1.12 (3, d, $J$ = 7 Hz, $CHCH_3$), 1.66 (3, s, $O(C)C(C)CH_3$), 2.83 (2, m, $OCH_2CH_2$), 3.17 (1, q, $J$ = 1 Hz, CHCO), 4.17 (2, m, $OCH_2$), 7.30 (4, m, aromatic protons), 8.60 (1, s, NH), 10.12 (1, s, COOH).

**1,3,4,9-Tetrahydro-1-ethyl-3-methylpyrano[3,4-*b*]indole-1-acetic Acids (19 and 20).** A mixture of $\alpha$-methyltryptophol[8] (12.5 g), ethyl propionylacetate (11.4 ml), and *p*-TSA (500 g) in benzene (200 ml) was refluxed for 3 hr. The usual work-up procedure gave an oil (19 g). Elution from a silica gel column with hexane–$Me_2CO$ (10:1) gave the ethyl ester of **20**, mp 99–101° (3.3 g). Hydrolysis with KOH in MeOH afforded **20** (1.65 g): mp 147–148°; nmr ($CDCl_3$) $\delta$ 0.96 (3, t $J$ = 7 Hz, $CH_2CH_3$), 1.48 (3, d, $J$ = 6.5 Hz, $CHCH_3$), 2.00 (2, m, $CH_2CH_3$), 2.71 (2, m, $CH_2CH$), 2.98 (2, m, $CH_2CO$), 4.17 (1, m, CHO), 7.12 (4, m, aromatic protons), 8.98 (1, s, NH), 10.35 (1, s, COOH). Further elution with hexane–$Me_2CO$ (10:1) gave the ethyl ester of **19** as an oil. Hydrolysis with KOH in MeOH gave **19** (1.61 g): mp 145–146°; nmr ($CDCl_3$) $\delta$ 0.60 (3, t, $J$ = 7 Hz, $CH_2CH_3$), 1.31 (3, d, $J$ = 6 Hz, $CHCH_3$), 2.10 (2, q, $J$ = 7 Hz, $CH_2CH_3$), 2.58 (2, m, $CH_2CH$), 2.72–3.00 (2, m, $CH_2COO$), 4.15 (1, m, CHO), 7.26 (4, m, aromatic protons), 10.00 (1, s, COOH), 10.60 (1, s, NH).

**1,3,4,9-Tetrahydro-1-ethyl-4-methylpyrano[3,4-*b*]indole-1-acetic Acids (21 and 22).** A mixture of $\beta$-methyltryptophol[9] (4.7 g), ethyl propionylacetate (4.2 g), and *p*-toluenesulfonic acid (250 mg) was refluxed in $C_6H_6$ for 3 hr. The solution was washed with 5% aqueous $NaHCO_3$, dried, and evaporated to give an oil (4.7 g). It was refluxed for 3 hr in a mixture of MeOH (200 ml), $H_2O$ (5 ml), and KOH (4.0 g) and after a conventional work-up procedure, a solid (3.5 g) was obtained. Fractional crystallization from $C_6H_6$–petroleum ether gave **22** (2.05 g): mp 153–154°; nmr ($CDCl_3$) $\delta$ 0.85 (3, t, $J$ = 7 Hz, $CH_2CH_3$), 1.36 (3, d, $J$ = 7 Hz, $CHCH_3$), 2.05 (2, q, $J$ = 7 Hz, $CH_2CH_3$), 3.01 (2, s, $CH_2COO$), 3.12 (1, m, $CHCH_3$), 3.63–4.08 (2, m, $CH_2O$), 7.20 (4, m, aromatic protons), 8.65 (1, s, NH), 10.10 (1, s, COOH). The mother liquors of **22** afforded a second crop of crystals which was recrystallized from $C_6H_6$ to give **21** (1.2 g), mp 132–133°. The nmr spectrum was identical with that of **22**. The mixture melting point of compounds **21** and **22** was depressed to 128°.

## References and Notes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