Synthesis and Pharmacological Properties of Substituted Cinnamohydroxamic Acids

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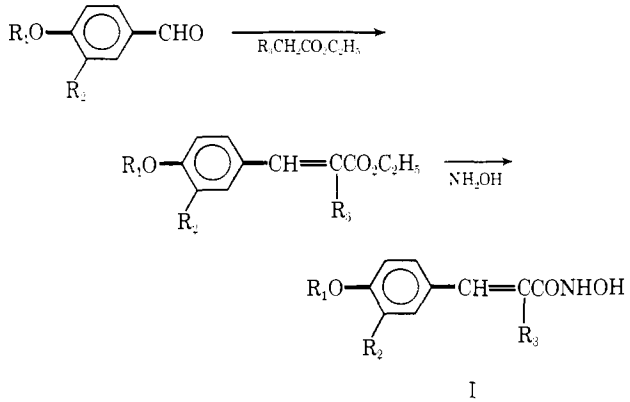
The synthesis of a large number of diversely substituted *p*-alkyloxycinnamohydroxamic acids is described, and their pharmacologic evaluation is reported. Some of these hydroxamic acids are shown to possess antiinflammatory and analgetic activity, though to a lower degree than the corresponding *p*-alkyloxyphenylacethydroxamic acids, and several of them manifested good antispasmodic activity *in vitro*.

In the course of our research on new nonsteroid antiinflammatory and analgetic agents, we showed the interesting activity of certain substituted arylacethydroxamic acids;[2-4] one of them, *p*-butoxyphenylacethydroxamic acid,[5] has since been found useful for the chemotherapy of rheumatoid diseases. In order to investigate further the relationship between molecular structure and antiinflammatory and analgetic activity, we undertook the study of substituted cinnamohydroxamic acids.

So far, only the nonsubstituted cinnamohydroxamic acid itself[6,7] has been investigated pharmacologically[8] and was found to possess CNS tranquillizing properties; an isostere of this acid, *i.e.*, β-3-pyridylacrylic acid, had also been investigated by one of us[9] and found to exhibit peripheral vasodilatatory and analeptic properties.

A. Chemistry.—The synthesis of compound I was effected according to the scheme below:



I

Where R_2, R_3 = H, the starting material was *p*-hydroxybenzaldehyde (**1–7**, **36**). Where R_3 = H and R_2 = F, Cl, Br, CH_3 (**8–13**, **23–29**), the starting material was *o*-fluorophenol, *o*-chlorophenol, *o*-bromophenol, and *o*-cresol, which were alkylated by means of the appropriate alkyl halide. The corresponding aldehydes were then obtained either by chloromethylation followed by a Sommelet reaction, or directly by a Rieche reaction[10] (formylation by dichloromethyl butyl ether in the presence of $TiCl_4$). Where R_2 = CH_3O and C_2H_5O (**14–22**, **31**, **32**, **35**), vanillin and 3-ethoxy-4-hydroxybenzaldehyde were the starting materials. For **30–32**, ethyl propionate was used in Claisen condensations with the appropriately substituted benzaldehyde; **33**, **34** were obtained starting from isovanillin. Table I lists the cinnamohydroxamic acids thus synthesized.

Experimental Section

Pharmacology.—As the products are insoluble in H_2O, they were administered orally in a mucilage of 1% tragacanth gum.

Antiinflammatory Properties.—For the two tests, 3 dose levels of each compound are studied. Activity was established using at least 25 male rats per dose.

Carrageenin-induced abscess test was performed according to the technique of Benitz and Hall.[11]

Cotton Pellet Granuloma Test.—We used a modified Meier test,[12] the treatment by antiphlogistic agents starting 3 days after insertion of the pellets. This procedure permits an assessment of the antiphlogistic potency in chronic inflammatory processes and gives more precise information on the activity on tissular reaction of the tested compound.

Analgetic Activities.—Two methods were used to measure the analgetic activities. Five dose levels of each compound were studied in groups of 10 male animals.

Pain Induced by Chemical Stimulus.—The Siegmund test[13] was modified following the recommendations of Hendershot and Forsaith[14] and Grimshaw and D'Arcy.[15] AcOH (75 mg/kg) was used instead of phenylbenzoquinone.

Pain induced by pressure on the inflamed paw of the Wistar rat[16] was obtained by means of a new type of apparatus (Ugo Basile, Milano, Italy) which gives the response in grams instead of millimeters.

Antipyretic Activity.—Fever was induced in rabbits by intravenous injection of antigonococcal vaccine (twice with 10^8

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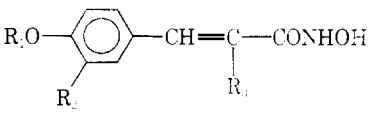
TABLE I



Compd	R_1	R_2	R_3	Mp, °C[a]	Formula[b]
1	CH_3	H	H	138–140 (D)	$C_{10}H_{11}NO_3$
2	C_2H_5	H	H	145–146 (D)	$C_{11}H_{13}NO_3$
3	*n*-C_3H_7	H	H	133–135 (D)	$C_{12}H_{15}NO_3$
4	*n*-C_4H_9	H	H	144–145.5 (D)	$C_{13}H_{17}NO_3$
5	*i*-C_4H_9	H	H	142–143 (D)	$C_{13}H_{17}NO_3$
6	*n*-C_5H_{11}	H	H	141.5–142.5 (D)	$C_{14}H_{19}NO_3$
7	*i*-C_5H_{11}	H	H	140.5–142 (D)	$C_{14}H_{19}NO_3$
8	CH_3	Cl	H	135–137 (A)	$C_{10}H_{10}ClNO_3$
9	C_2H_5	Cl	H	139–141 (D)	$C_{11}H_{12}ClNO_3$
10	*n*-C_3H_7	Cl	H	139.5–142 (D)	$C_{12}H_{14}ClNO_3$
11	*n*-C_4H_9	Cl	H	136–137 (D)	$C_{13}H_{16}ClNO_3$
12	*n*-C_5H_{11}	Cl	H	124–125.5 (E)	$C_{14}H_{18}ClNO_3$
13	*i*-C_5H_{11}	Cl	H	126.5–128 (D)	$C_{14}H_{18}ClNO_3$
14	C_2H_5	CH_3O	H	167–169 (A)	$C_{12}H_{15}NO_4$
15	*n*-C_3H_7	CH_3O	H	147–148.5 (A)	$C_{13}H_{17}NO_4$
16	*n*-C_4H_9	CH_3O	H	133–135 (A)	$C_{14}H_{19}NO_4$
17	*n*-C_5H_{11}	CH_3O	H	113–115 (D)	$C_{15}H_{21}NO_4$
18	CH_2=CH—CH_2	CH_3O	H	150.5–152.5 (D)	$C_{13}H_{15}NO_4$
19	CH_3	CH_3O	H	165–166 (D)	$C_{11}H_{13}NO_4$
20	*n*-C_3H_7	C_2H_5O	H	147–148.5 (A)	$C_{14}H_{19}NO_4$
21	*n*-C_4H_9	C_2H_5O	H	126–128.5 (A)	$C_{15}H_{21}NO_4$
22	*n*-C_5H_{11}	C_2H_5O	H	113–114.5 (A)	$C_{16}H_{23}NO_4$
23	CH_3	CH_3	H	139–140 (D)	$C_{11}H_{13}NO_3$
24	C_2H_5	CH_3	H	134.5–136 (D)	$C_{12}H_{15}NO_3$
25	*n*-C_3H_7	CH_3	H	122–125.5 (D)	$C_{13}H_{17}NO_3$
26	*n*-C_4H_9	CH_3	H	124–126 (D)	$C_{14}H_{19}NO_3$
27	*n*-C_5H_{11}	CH_3	H	99–100.5 (D)	$C_{15}H_{21}NO_3$
28	*n*-C_4H_9	Br	H	140–142 (A)	$C_{13}H_{16}BrNO_3$
29	*n*-C_4H_9	F	H	135–136 (A)	$C_{13}H_{16}FNO_3$
30	*n*-C_5H_{11}	H	CH_3	140–142.5 (E)	$C_{15}H_{21}NO_3$
31	*n*-C_4H_9	CH_3O	CH_3	137–139 (E)	$C_{15}H_{21}NO_4$
32	*n*-C_4H_9	C_2H_5O	CH_3	139.5–140.5 (E)	$C_{16}H_{23}NO_4$
33	CH_3	*n*-C_4H_9O	H	146–148 (A)	$C_{14}H_{19}NO_4$
34	CH_3	*n*-$C_5H_{11}O$	H	137–139 (A)	$C_{15}H_{21}NO_4$
35	CH_2=$CHCH_2$	C_2H_5O	H	99–100 (E)	$C_{14}H_{17}NO_4$
36	CH_2=$CHCH_2$	H	H	132.5–134 (E)	$C_{12}H_{13}NO_3$

[a] Melting point of analytical sample (uncorrected). [b] Crystallization: *i*-Pr_2O (E), EtOAc (A), or 1,2-dichloroethane (D). [c] All compounds analyzed satisfactorily for C, H, N.

bacteria/kg). Otherwise the technique was the same as that of Baker.[17]

Antispasmodic Activity.—Classical *in vitro* tests were used. The compounds were put into contact with guinea pig ileum for 2 min. The inhibition of spasms induced by histamine, AcCh and $BaCl_2$ was studied. At least three determinations were performed for each compound.

B. Chemistry.—Melting points were determined in open capillary tubes on a Tottoli apparatus and are uncorrected, as are boiling points. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ± 0.4% of the theoretical values. Microanalyses were performed by Janssen Pharmaceutica, Beerse (Ant.), Belgium. An example of synthesis is given below for 4-*n*-butoxy-3-fluorocinnamohydroxamic acid.

4-*n*-Butoxy-3-fluorobenzyl Chloride.—4-*n*-Butoxy-3-fluorobenzene (141 g), 100 ml of aqueous CH_2O (40%) and 300 ml of glacial AcOH were heated for 5 hr at 90° with stirring. At this temperature dry HCl was passed into the reaction mixture. After cooling, the organic layer was removed and the aqueous layer extracted twice in C_6H_6. The combined organic layers were washed with ice–water saturated with NaCl, dried ($MgSO_4$) and fractionated; 137 g of product was obtained (76%), bp 130–135° (1 mm) $n^{25}D$ 1.5080. *Anal.* ($C_{11}H_{14}ClFO$) C, H.

4-*n*-Butoxy-3-fluorobenzaldehyde.—In a 2-l. flask were placed 137 g of 4-*n*-butoxy-3-fluorobenzyl chloride, 137 g of hexamethylenetetramine, 300 ml of glacial AcOH and 300 ml of H_2O. The mixture was heated under reflux for 2 hr, 200 ml of HCl was added and refluxing continued for 15 min. After cooling the mixture was extracted (Et_2O). The organic layer was washed (H_2O), 10% aqueous Na_2CO_3, H_2O), and the Et_2O extract dried ($MgSO_4$) and distilled. On vacuum distillation, the residual liquid gave 94.7 g (75%) of an oil: bp 125–126° (0.8 mm), $n^{25}D$ 1.5038. *Anal.* ($C_{11}H_{13}FO_2$) C, H.

Ethyl 4-*n*-Butoxy-3-fluorocinnamate. Dry xylene (200 ml) and 14 g of clean Na were heated until the Na had melted, and broken up by stirring into small particles. The xylene was poured off and 245 ml of absolute EtOAc containing 2 ml of absolute EtOH was added. The mixture was cooled to 0–10° and 94.7 g of 4-*n*-butoxy-3-fluorobenzaldehyde was added slowly with stirring. After all the Na had disappeared (2 hr), 50 ml of glacial AcOH and H_2O were added. The mixture was extracted with EtOAc and the organic layer washed with 6 *N* HCl and dried ($MgSO_4$). The solvent was evaporated and the remaining liquid distilled. The product weighed 86 g (69%), bp 152–158° (0.3 mm). *Anal.* ($C_{15}H_{19}FO_3$) C, H.

4-*n*-Butoxy-3-fluorocinnamahydroxamic Acid (29).—$NH_2OH \cdot HCl$ (7.5 g) in 100 ml of MeOH was mixed with a solution of 13.5 of NaOH in 100 ml of MeOH. To the filtered solution 28.2 g of ethyl 4-*n*-butoxy-3-fluorocinnamate was added dropwise with stirring and the mixture refluxed for 1 hr, cooled, poured into ice, and acidified with 20% HCl. The white solid was washed (H_2O) and recrystallized (EtOAc): yield, 71%, mp 134–136°.

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TABLE II

Compd	LD_{50}, mg/kg mouse *per os*[a]	Antiinflammatory Act.[b]	Antispasmodic Act.[c] Hist	AcCh	$BaCl_2$
1	2000 (1504–2660)	0	>14	>14	>14
2	1700 (1164–2482)	0	>14	>14	>14
3	1670 (1347–2071)	0	14	>14	>14
4	1620 (1246–2106)	0	14	>14	3.5
5		0	>14	>14	>14
6	2710 (2134–3442)	0	14	>14	
7		0	14	>14	14
8		0	14	>14	
9	1530 (1085–2157)	+			
10	1740 (1349–2245)	0	7	14	14
11		0			
12		0	>14	>14	>14
13	2580 (1665–3999)	0	>14	>14	>14
14	810 (675–972)	++[d]			
15	1600 (1103–2320)	++[d]	14	>14	
16	1380 (1160–1642)	0	7	>14	14
17	±4000	0	7	14	
18	1700 (1371–2108)	0	7	>14	
19	830 (565–1220)	++	>14	14	
20	1980 (1523–2574)	0	1.4	1.4	>14
21	1630 (1315–2021)	+	1.4	1.4	>14
22	2400 (1778–3240)	+	1.4	14	>14
23	1600 (1260–2032)	+	>14	>14	
24	2300 (1679–3150)	0	7	14	
25	1950 (1585–2399)	0	7	14	
26	2150 (1680–2752)	0	7	7	7
27		0	>14	>14	>14
28	±4000	0	>14	14	>14
29	±2000	0	>14	7	
30	2400 (1818–3168)	0	>14	>14	
31	±3000	0	14	>14	
32	±4000	0	14	>14	
33	2450 (1992–3013)	+++[d]	14	14	14
34	3300 (2237–4807)	++	14	14	14
35	>4000	0			
36		0	14	>14	>14
Droxaryl	8000	+++	14	14	14

[a] LD_{50} values are given in mg/kg; ( ) confidence limits to 95%. [b] Phenylbutazone was taken as standard (0 = absence of activity, + = from 0 to 0.25 of phenylbutazone, ++ = 0.25 to 0.5 of phenylbutazone, and +++ = 0.5 to 1 of phenylbutazone. [d] These compounds show a good activity in the Abscess test; they are inactive in the Granuloma test. [c] Values expressed in μg/ml corresponding to doses necessary to obtain total inhibition of the spasm (Hist = histamine, AcCh = acetylcholine). [e] Droxaryl is *p*-butoxyphenylacethydroxamic acid.

Results and Discussion

Table II shows that, at variance with observations with arylacethydroxamic acids bearing the same nuclear substituents, the antiinflammatory activity of the present cinnamohydroxamic acids is on the whole rather weak. Only **14**, **15**, **19**, **33**, and **34** display a noteworthy activity but still lower than that of the phenylbutazone standard. Neither lengthening nor branching of the alkyl chain R_1, nor the introduction of various radicals R_2, enhanced the antiinflammatory potency. *p*-Butoxyphenylacethydroxamic acid[4] exhibited a high activity in the granuloma test, whereas **14**, **15**, and **33** were completely inactive in that respect.

In regard to relationships between antiinflammatory activity and the nature of the chain bearing CO_2H, we also prepared and tested a series of *para*-substituted β-phenylpropionhydroxamic acids; these derivatives showed no antiinflammatory properties. This is also the case for the corresponding benzhydroxamic acids. It thus appears indispensable for antiinflammatory activity that the hydroxamic acid function be separated from the benzene ring by only a single C.

The hypothesis of a biochemical cellular receptor common to the various antiinflammatory agents had already been advanced by different authors[18,19] Molecular models show that *p*-butoxyphenylacethydroxamic acid could interact with a receptor having the same characteristics as the one Shen proposed for indomethacin.[18] But in the cinnamic acid series, the introduction of a double bond endows the molecule with a quite different geometry, and it is more difficult to fit this type of molecule to the proposed receptor model.

Analgetic activity in the cinnamohydroxamic acids is likewise relatively low compared with the corresponding *para*-substituted arylacethydroxamic acids, and so is the antipyretic activity, with the exception of **18** which is twice as active as acetylsalicylic acid.

Several cinnamohydroxamic acids have manifested a good antispasmodic activity *in vitro*.

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