(2) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972; G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, N.Y., 1972; L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley-Interscience, New York, N.Y., 1972.
(3) See, for example (a) H. G. Floss, *Lloydia*, **35**, 399 (1972); (b) J. B. Grutzner, *ibid.*, **35**, 375 (1972); (c) L. L. Martin, C.-j. Chang, H. G. Floss, J. A. Mabe, E. W. Hagaman, and E. Wenkert, *J. Am. Chem. Soc.*, **94**, 8942 (1972); (d) R. D. Johnson, A. Haber, and K. L. Rinehart, Jr., *ibid.*, **96**, 3316 (1974); (e) T. C. Feline, G. Mellows, R. B. Jones, and L. Phillips, *J. Chem. Soc., Chem. Commun.*, 63 (1974); (f) G. McInnes, D. G. Smith, J. A. Walter, L. C. Vining, and J. J. C. Wright, *ibid.*, 282 (1974); (g) M. Tanabe and K. T. Suzuki, *ibid.*, 445 (1974); (h) A. R. Battersby, M. Ihara, E. McDonald, and J. R. Stephenson, *ibid.*, 458 (1974); (i) J. W. Westley, R. H. Evans, Jr., G. Harvey, R. G. Pitcher, D. L. Pruess, A. Stempel, and J. Berger, *J. Antibiot.*, **27**, 288 (1974); and (K) J. R. Hanson, T. Marten, and M. Siverns, *J. Chem. Soc., Perkin Trans. 1*, 1033 (1974).
(4) See, for example (a) E. Wenkert, C.-j. Chang, D. W. Cochran, and R. Pellicciari, *Experientia*, **28**, 377 (1972); (b) E. Wenkert, C.-j. Chang, A. O. Clouse, and D. W. Cochran, *J. Chem. Soc., Chem. Commun.*, 961 (1972); (c) A. G. McInnes, D. G. Smith, C. K. Wat, L. C. Vining, and J. J. C. Wright, *ibid.*, 281 (1974); (d) K. Nakanishi, V. P. Gullo, I. Miura, T. R. Govindachari, and N. Viswanathan, *J. Am. Chem. Soc.*, **95**, 6473 (1973); (e) F. J. Schmitz and F. J. McDonald, *Tetrahedron Lett.*, 2541 (1974); and (f) E. Wenkert, J. S. Bindra, C.-j. Chang, D. W. Cochran, and F. M. Schell, *Acc. Chem. Res.*, **7**, 46 (1974).
(5) (a) N. J. Bach, H. E. Boaz, E. C. Kornfeld, C.-j. Chang, H. G. Floss, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, **39**, 1272 (1974); (b) F. I. Carroll and C. G. Moreland, *J. Chem. Soc., Perkin Trans. 1*, 374 (1974); (c) T. E. Walker, H. P. C. Hogenkamp, T. E. Needham, and N. A. Matwiyoff, *J. Chem. Soc., Chem. Commun.*, 85 (1974); (d) A. A. Gallo and H. Z. Sable, *J. Biol. Chem.*, **249**, 1382 (1974); (e) L. Simeral and G. E. Maciel, *Org. Magn. Reson.*, **6**, 226 (1974); (f) N. S. Bhacca, D. D. Giannini, W. S. Jankowski, and M. E. Wolff, *J. Am. Chem. Soc.*, **95**, 842 (1973); and (g) J. Okada and T. Esaki, *Yakugaku Zasshi*, **93**, 1014 (1973).
(6) (a) P. Lazzeretti and F. Taddei, *Org. Magn. Reson.*, **3**, 283 (1971); (b) G. Miyajima, Y. Sasaki, and M. Suzuki, *Chem. Pharm. Bull.*, **19**, 230 (1971); (c) G. Miyajima, Y. Sasaki, and M. Suzuki, *ibid.*, **20**, 429 (1972); and (d) Y. Sasaki, *ibid.*, **21**, 1901 (1973).
(7) (a) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966); (b) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Am. Chem. Soc.*, **91**, 7445 (1969).
(8) (a) A. R. Tarpley, Jr., and J. H. Goldstein, *J. Mol. Spectrosc.*, **39**, 275 (1971); (b) A. R. Tarpley, Jr., and J. H. Goldstein, *J. Am. Chem. Soc.*, **93**, 3573 (1971); and (c) F. J. Weigert and J. D. Roberts, *ibid.*, **89**, 2967 (1967).
(9) (a) P. C. Lauterbur, *J. Chem. Phys.*, **43**, 360 (1965); (b) A. Mathias and V. M. S. Gil, *Tetrahedron Lett.*, 3163 (1965); and (c) R. J. Pugmire and D. M. Grant, *J. Am. Chem. Soc.*, **90**, 697 (1968).
(10) H. L. Retcofsky and R. A. Friedel, *J. Phys. Chem.*, **72**, 2619 (1968).
(11) C.-j. Chang, H. G. Floss, and G. E. Peck, unpublished data.
(12) R. Hagen and J. D. Roberts, *J. Am. Chem. Soc.*, **91**, 4504 (1969).
(13) W. J. Horsley and H. Sternlicht, *J. Am. Chem. Soc.*, **90**, 3738 (1968).
(14) W. Adam, A. Grimison, and G. Rodriguez, *J. Chem. Phys.*, **50**, 645 (1969).
(15) R. J. Pugmire and D. M. Grant, *J. Am. Chem. Soc.*, **90**, 697 (1968).

Antiinflammatory β-Arylamidoacrylic Acids

Robert T. Buckler,* Harold E. Hartzler,

Medicinal Chemistry Department

and Barrie M. Phillips

Toxicology Department, The Research Division, Miles Laboratories, Inc., Elkhart, Indiana 46514. *Received June 27, 1974*

A series of 34 β-arylamidoacrylic acids was prepared and examined for antiinflammatory activity. These compounds are vinylogous carbamic acids, and several displayed activity equal to phenylbutazone in the rat pleural effusion model. Highest activity was associated with structures bearing halogen and cyano substituents. Amides were inactive.

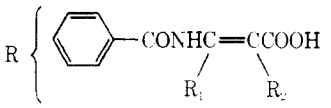
We became interested in the title compounds for two reasons. They appear as partial structures in a number of antiinflammatory compounds such as the fenamates and the related benzamido benzoic acids.[1,2] And they satisfy the rudimentary structural requirements suggested for certain nonsteroidal antiinflammatory acids: an aryl group attached to a nonbasic nitrogen atom that is separated from the carboxyl group by one or two atoms.[3,4] Because of its simplicity, we felt that the structure of these compounds might be close to the minimum geometry and constitution necessary for such activity.

Carbamic acids are unstable, they decompose spontaneously with loss of carbon dioxide. The few that are known are extensively stabilized by hydrogen bonding.[5] Since double bonds transmit the electrical effects causing this instability, there was some doubt that vinylogous carbamic acids would be stable enough to be useful, even though one of them had been casually characterized earlier.[6] Nevertheless, when prepared these acids turned out to be stable to all but high temperatures and strong acids. They all decarboxylate at the melting point.

Chemistry. The arylamidoacrylic acids III were prepared as outlined in Scheme I (see Table I for details). The starting β-aminoacrylate esters I were made from the appropriate β-keto esters in the usual way.[7] Ethyl esters were preferred but methyl and *tert*-butyl esters were used when necessary. The acylation of the β-amino esters to give the β-amido esters II (see Table II for details) was uneventful except when the starting β-amino ester possessed an α-hydrogen atom (I, R_2 = H). In these cases the reaction proceeded to give both an N-acylated product (II, R_2 = H) and a C-acylated product (IV). The isomer ratio varied according to the reaction conditions but it was roughly unity for reactions in ether at room temperature. The isomer mixtures were easily separated by fractional crystallization or column chromatography on alumina or Florisil.

The ^{1}H NMR spectra of the N-acylated isomers displayed the vinyl proton at R_2 as a singlet in the range 290–310 Hz ($CDCl_3$). This peak was absent from the spectra of the C-acylated isomers. The infrared spectra of the C-acyl-

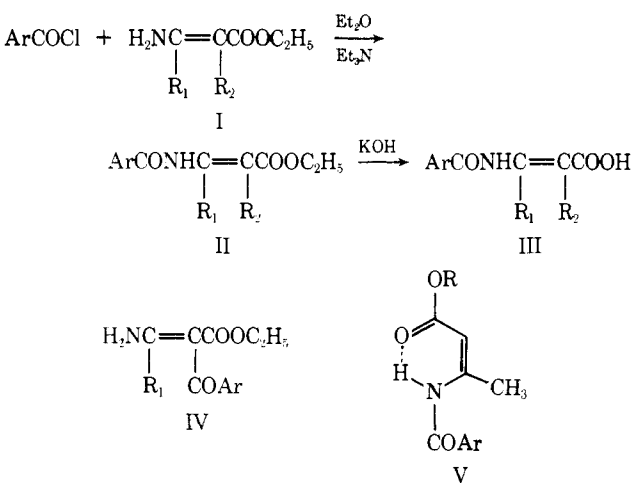
Table I. β-Arylamidoacrylic Acids



No.	R	R_1	R_2	Mp, °C[a]	% yield	Formula	Recrystn solvent	Analyses	AI[b]
1	H	CH_3	H	140[c]	48	$C_{11}H_{11}NO_3$	C_6H_6–ligroine	C, H, N	3.0
2	4-F	CH_3	H	155	35	$C_{11}H_{10}FNO_3$	MeOH–H_2O	C, H, N	7.1
3	3-Cl	CH_3	H	146	23	$C_{11}H_{10}ClNO_3$	C_6H_6	C, H, N	3.4
4	4-Cl	CH_3	H	149	5	$C_{11}H_{10}ClNO_3$	C_6H_6	C, H, N	9.1
5	3,4-Cl_2	CH_3	H	167	11[d]	$C_{11}H_9Cl_2NO_3$	C_6H_6	C, H, N	[e]
6	3-Br	CH_3	H	149	35[d]	$C_{11}H_{10}BrNO_3$	C_6H_6	C, H, N	1.9
7	4-Br	CH_3	H	164	7	$C_{11}H_{10}BrNO_3$	MeOH	C, H, N	7.1
8	4-CH_3	CH_3	H	150	37	$C_{12}H_{13}NO_3$	C_6H_6	C, H, N	9.1
9	4-C_2H_5	CH_3	H	111	11	$C_{13}H_{15}NO_3$	C_6H_6	C, H, N	6.7
10	4-*i*-Pr	CH_3	H	114	12	$C_{14}H_{17}NO_3$	Ligroine	C, H, N	9.2
11	4-*t*-Bu	CH_3	H	136	15	$C_{15}H_{19}NO_3$	Me_2CO–ligroine	C, H, N	5.2
12	4-CN	CH_3	H	177	2	$C_{12}H_{10}N_2O_3$	MeOH	C, H, N	9.0
13	4-CH_3O	CH_3	H	147	13	$C_{12}H_{13}NO_4$	C_6H_6	C, H, N	1.3
14	4-NMe_2	CH_3	H	142	37	$C_{13}H_{16}N_2O_3$	MeOH	C, H, N	1.0
15	H	CH_3	CH_3	155	47	$C_{12}H_{13}NO_3$	EtOAc–C_6H_6	C, H, N	3.7
16	4-Cl	CH_3	CH_3	171	21	$C_{12}H_{12}ClNO_3$	C_6H_6	C, H, N	10.0
17	3,4-Cl_2	CH_3	CH_3	183	44	$C_{12}H_{11}Cl_2NO_3$	*i*-PrOH	C, H, N	4.8
18	3-Br	CH_3	CH_3	172	37	$C_{12}H_{12}BrNO_3$	C_6H_6	C, H, N	5.0
19	3,5-Br_2	CH_3	CH_3	182	42	$C_{12}H_{11}Br_2NO_3$	MeOH	C, H, N	1.6
20	3-I	CH_3	CH_3	171	52	$C_{12}H_{12}INO_3$	MeOH[f]	C, H, N	5.8
21	4-I	CH_3	CH_3	184	57	$C_{12}H_{12}INO_3$	MeOH[f]	C, H, N	5.2
22	4-CH_3O	CH_3	CH_3	150	58	$C_{13}H_{15}NO_4$	MeOH	C, H, N	4.5
23	4-Pyridyl	CH_3	CH_3	170	19	$C_{11}H_{10}N_2O_3$	EtOAc	H, N; C[g]	2.8
24	4-*i*-Pr	CH_3	CH_3	141	36	$C_{15}H_{19}NO_3$	MeOH–H_2O	C, H, N	1.7
25	4-*t*-Bu	CH_3	CH_3	167	40	$C_{16}H_{21}NO_3$	EtOAc	C, H, N	10.1
26	H	CH_3	C_2H_5	127	38	$C_{13}H_{15}NO_3$	Me_2CO–ligroine	C, H, N	7.1
27	4-Br	CH_3	C_2H_5	150	5	$C_{13}H_{14}BrNO_3$	EtOAc	C, H, N	11.0
28	4-*i*-Pr	CH_3	C_2H_5	112	33	$C_{16}H_{21}NO_3$	C_6H_6–ligroine	H, N; C[h]	4.2
29	H	C_6H_5	H	152	57	$C_{16}H_{13}NO_3$	MeOH–H_2O	C, H, N	1.5
30	H	CH_2COOH	H	174	50	$C_{12}H_{11}NO_5$	EtOAc	H, N; C[i]	1.0
31	H	$-(CH_2)_3-$		169	45	$C_{14}H_{13}NO_3$	EtOAc	C, H, N	4.3
32	4-Br	$-(CH_2)_4-$		180	50	$C_{14}H_{14}BrNO_3$	DMF–H_2O	C, H, N	5.2
33	4-CN	$-(CH_2)_3-$		198	45	$C_{15}H_{11}N_2O_3$	DMF–H_2O	C, H, N	14.2
34	4-Pyridyl	$-(CH_2)_4-$		197	2	$C_{13}H_{11}N_2O_3$	DMF–MeOH	C, H, N	4.3

[a] These compounds effervesce upon melting. [b] Activity index = ten times the ratio of the % reduction of the mean pleural exudate volumes of the test compound at 1 mmol/kg over 1 mmol/kg of aspirin. AI for phenylbutazone = 14.3. [c] Reference 6 reports mp 139°. [d] From cleavage of the *tert*-butyl ester. [e] Not tested, insufficient compound. [f] Washed with methanol. [g] C: calcd, 59.99; found, 59.23. [h] C: calcd, 69.80; found, 70.25. [i] C: calcd, 57.83; found, 57.12.

Scheme I



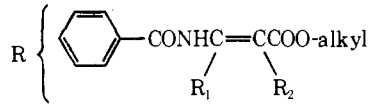
ated isomers displayed the typical NH_2 stretching absorptions at 3480 (s) and 3300 cm^{-1} (m) while the N-acylated compounds exhibited a moderately strong NH band at 3250 cm^{-1}.[8]

Both *E* and *Z* isomers are possible in structures II and III. However, only one geometrical isomer is observed, the strongly hydrogen bonded *Z* isomer V. This assignment is supported by the ^1H NMR spectra of the N-acylated β-amino esters. A very broad, one-proton multiplet is found centered at about 723 Hz in the esters ($CDCl_3$) and about 630 Hz in the acids (DMSO-d_6) indicative of bonded N–H. No peak for free N–H is seen. Also, the parent ester of the series (II, Ar = phenyl; R_1 = CH_3; R_2 = H) had λ_{max} 295 nm [ε 15,900 ($CHCl_3$)]. This is precisely as calculated for the *Z* isomer from prior studies on the uv spectra of vinylogous amides.[9]

As a kind of structure proof, one of the C-acylated isomers was hydrolyzed, *tert*-butyl β-amino-α-(3-bromobenzoyl)crotonate (**68**). When treated with CH_2Cl_2–HCl, it gave β-amino-3-bromocrotonophenone (**69**) via the spontaneous decarboxylation of the intermediate free acid.

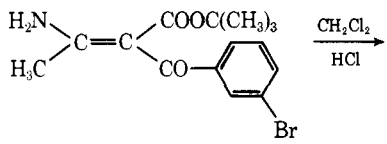
The saponification of the N-acylated β-aminoacrylates gave the expected β-arylamidoacrylic acids III in fair

Table II. β-Arylamidoacrylate Esters

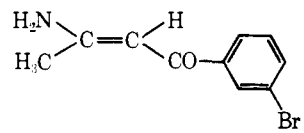


No.	R	R_1	R_2	Ester[a]	Mp, °C	% yield	Formula	Recrystn solvent	Analyses
35	4-F	CH_3	H	A	102	67	$C_{12}H_{12}FNO_3$	MeOH	C, H, N
36	3-Cl	CH_3	H	A	87	42	$C_{12}H_{12}ClNO_3$	Ligroine	H, N; C[b]
37	4-Cl	CH_3	H	A	129	63	$C_{12}H_{12}ClNO_3$	C_6H_6–ligroine	C, H, N
38	3,4-Cl_2	CH_3	H	C	102	7	$C_{15}H_{17}Cl_2NO_3$	MeOH–H_2O	C, H, N
39	3-Br	CH_3	H	C	103	29	$C_{15}H_{18}BrNO_3$	MeOH	C, H, N
40	4-Br	CH_3	H	B	104	37	$C_{13}H_{14}BrNO_3$	MeOH	C, H, N
41	4-CH_3	CH_3	H	B	67	42	$C_{14}H_{14}NO_3$	Ligroine	H, N; C[c]
42	4-C_2H_5	CH_3	H	A	63[d]	57	$C_{14}H_{17}NO_3$	MeOH	C, H, N
43	4-*i*-Pr	CH_3	H	A	69	58	$C_{15}H_{19}NO_3$	Ligroine	C, H, N
44	4-*t*-Bu	CH_3	H	B	74	42	$C_{17}H_{23}NO_3$	MeOH–H_2O	C, H, N
45	4-CN	CH_3	H	A	173	47	$C_{13}H_{12}N_2O_3$	MeOH	C, H, N
46	4-CH_3O	CH_3	H	B	111	26	$C_{14}H_{17}NO_4$	C_6H_6–ligroine	C, H, N
47	4-NMe_2	CH_3	H	A	117	27	$C_{14}H_{18}N_2O_3$	MeOH	C, H, N
48	H	CH_3	CH_3	B	96	59	$C_{14}H_{17}NO_3$	Ligroine	H, N; C[e]
49	4-Cl	CH_3	CH_3	B	128	80	$C_{14}H_{16}ClNO_3$	EtOH	C, H, N
50	3,4-Cl_2	CH_3	CH_3	B	146	65	$C_{14}H_{15}Cl_2NO_3$	EtOH	C, H, N
51	3-Br	CH_3	CH_3	B	105	60	$C_{14}H_{16}BrNO_3$	EtOH	H, N; C[f]
52	3,5-Br_2	CH_3	CH_3	B	99	68	$C_{14}H_{15}Br_2NO_3$	EtOH	C, H, N
53	3-I	CH_3	CH_3	B	105	65	$C_{14}H_{16}INO_3$	EtOH	C, H
54	4-I	CH_3	CH_3	B	128	72	$C_{14}H_{16}INO_3$	EtOH	C, H, N
55	4-CH_3O	CH_3	CH_3	B	97	51	$C_{15}H_{19}NO_4$	EtOH	H, N; C[g]
56	4-Pyridyl	CH_3	CH_3	B	139	73	$C_{13}H_{16}N_2O_3$	EtOH–H_2O	C, H, N
57	4-*i*-Pr	CH_3	CH_3	B	61	59	$C_{17}H_{23}NO_3$	EtOH–H_2O	C, H, N
58	4-*t*-Bu	CH_3	CH_3	B	92	66	$C_{18}H_{25}NO_3$	EtOH	C, H, N
59	H	CH_3	C_2H_5	B	75	70	$C_{15}H_{19}NO_3$	EtOH–H_2O	C, H, N
60	4-Br	CH_3	C_2H_5	B	83	89	$C_{15}H_{18}BrNO_3$	EtOH	C, H, N
61	4-*i*-Pr	CH_3	C_2H_5	B	Oil	44	$C_{13}H_{14}NO_3$	*h*	C, H, N
62	H	C_6H_5	H	B	*i*	46	$C_{18}H_{17}NO_3$		C, H, N
63	H	CH_2COOEt	H	B	79	25	$C_{16}H_{19}NO_5$	Ligroine	C, H, N
64	H	$-(CH_2)_4-$		B	104	70	$C_{16}H_{19}NO_3$	EtOH–H_2O	C, H, N
65	4-Br	$-(CH_2)_4-$		B	119	35	$C_{17}H_{18}N_2O_3$	EtOH	C, H, N
66	4-CN	$-(CH_2)_4-$		B	81	48	$C_{16}H_{18}BrNO_3$	EtOH	C, H, N
67	4-Pyridyl	$-(CH_2)_4-$		B	118	50	$C_{15}H_{18}N_2O_3$	EtOH	C, H, N

[a] A = methyl; B = ethyl; C = *tert*-butyl. [b] C: calcd, 56.80; found, 57.31. [c] C: calcd, 67.99; found, 67.50. [d] Bp 153° (0.5 mm). [e] C: calcd, 67.99; found, 68.96. [f] C: calcd, 51.55; found, 50.89. [g] C: calcd, 64.96; found, 65.57. [h] Chromatographed on Florisil, eluted with benzene-ligroine. [i] Bp 185–195° (0.7 mm).



68

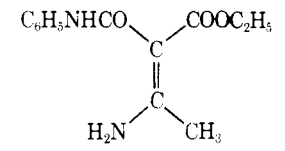


69

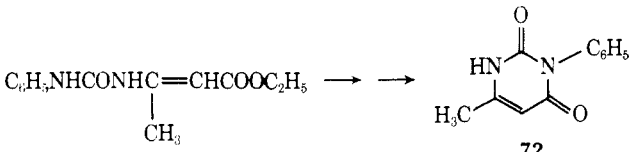
yields. A previous report had mentioned that the saponification of ethyl β-benzamidocrotonate with KOH–ethanol gave β-benzamidocrotonic acid in only 7% yield. The major products were potassium benzoate and ethyl benzoate in 27 and 47% yield, respectively.[6] Now under these conditions, ethyl benzoate can arise only by the attack of the lyate ion of the solvent on the amide carbonyl group of the enamido ester II. We repeated this reaction using 2-propanol as the solvent since this alcohol has little tendency to form the lyate ion. With these conditions the yield of β-benzamidocrotonic acid 1 was increased to 48%. This KOH–2-propanol method was then used to make most of the acids listed in Table I. However, esters with deactivating aromatic substitutents still gave very poor yields of acids when saponified; hydrolysis of the enamide group became the major reaction. We avoided this side reaction by preparing the analogous *tert*-butyl esters and cleaving them with CH_2Cl_2–HCl.[10]

In one case we used phenyl isocyanate as the acylating agent. When reacted with ethyl β-aminocrotonate, it too gave both a C- and a N-acylated product, **70** and **71**. The N isomer was saponified as usual, but when acidified, the β-phenylureidocrotonic acid that resulted cyclized spontaneously to 6-methyl-3-phenyluracil (**72**).[11]

Pharmacology. The antiinflammatory activity of these compounds was measured by their ability to protect rats against pleurisy induced by the irritants Evans Blue and carrageenan. The introduction of a mixture of these irritants (0.05 ml of a 0.316% aqueous solution) into the rat pleural cavity causes an inflammation which results in an increase in the volume of pleural fluid in the following 6 hr. This increase can be inhibited by a wide variety of antiinflammatory compounds including steroids and gold salts, and the inhibition is dose dependent.[12]



70



71 72

Each test compound was administered orally to six rats 1 hr before the irritants at a dose of 1 mmol/kg. Six hours after the irritants were given, the animals were killed and the volumes of their pleural fluid measured. These values were compared with those of control groups as well as those from animals treated with 1 mmol/kg doses of aspirin. The results are expressed as an activity index (AI) which is a value equal to ten times the ratio of the percent reduction of the mean pleural exudate volumes of the rats treated with the test compound at 1 mmol/kg divided by the percent reduction of the volumes for the animals treated with aspirin.

Discussion

Geometry, size, electronic state, and lipophilicity are the four principle factors that influence biological activity in a drug series where the basic functional elements are kept constant. This series was designed in such a way as to keep the overall geometry and chief functional elements unchanged while varying the aromatic substitutents and the substitutents about the double bond.

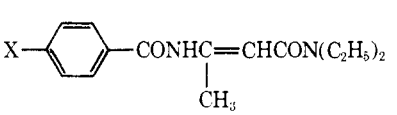
There is no obvious structure–activity pattern in this series, although some structural features do seem to be better than others. Halogens were the best aromatic substitutents and were more effective in the para than in the meta position. The equally high activity of the 4-cyano analogs **12** and **33** suggests that the biological response might be directly proportional to the degree of electron deficiency of the aromatic ring. Even so, two 4-alkyl-substituted compounds, **10** and **25**, were also somewhat active.

The substitution of alkyl groups for hydrogen at the α position (R_2 in Table I) had a slight but positive effect on the biological activity. Large alkyl groups such as C_2H_5 and $(CH_2)_4$ appear best.

Most of the compounds studied have a methyl group at the β-carbon atom (R_1 in Table I). Its replacement by phenyl in **29** gave an inactive compound.

There is no clear cut dependence of antiinflammatory activity on lipophilicity in this series. Still, in spite of exceptions like **19**, **21**, **24**, and **31**, activity is greater for the more lipophilic members of the set. The most active compound, the 4-cyanocyclohexyl analog **33**, does combine high lipophilicity with deactivation of the aromatic ring. The activity of **33** seems to indicate that our original hypothesis may be correct: structures like III may well represent a kind of minimum structural requirement for the antiinflammatory activity associated with arylalkanoic acids.

Three amides were tested. They are the 4-fluoro **73**, the 4-ethyl **74**, and the 4-chloro analog **75** of *N,N*-diethyl-β-benzamidocrotonamide VI. All were inactive.



VI

Experimental Section

All melting points are uncorrected and were determined with a Büchi capillary melting point apparatus. Analyses indicated by symbols were within ±0.4% of the calculated values.

β-Aminoacrylate Esters, I. These were made by refluxing the corresponding β-keto esters and equal volumes of pentane with 50 mg of *p*-toluenesulfonic acid while bubbling in a slow stream of ammonia with rapid stirring. A water separator was attached. Higher boiling hydrocarbon solvents were less efficient, probably due to lower solubility of ammonia. This procedure gave an 80% yield on a 2-mol scale of *tert*-butyl β-aminocrotonate: mp 37°; bp 60° (0.4 mm) (lit.[13] mp 37–39°). Heating for 36 hr was required. Two of the amino esters made in this way were new compounds: ethyl 3-amino-2-ethylcrotonate [mp 57° recrystallized from EtOH. Anal. Calcd for $C_8H_{15}NO_2$: N, 8.91. Found: N, 8.84] and diethyl 3-aminoglutaconate [bp 110–112° (0.2 mm). Anal. Calcd for $C_9H_{15}NO_5$: neut equiv 217. Found: 221].

Acylation of the β-Aminoacrylate Esters, I. The amino esters and 1 equiv of triethylamine or pyridine were heated to reflux in ether or tetrahydrofuran, 2 l./mol of ester. The acid chlorides, 1.05 equiv, were added dropwise over 1 hr. Heating was continued for a total of 24 hr. When cool, the solutions were filtered, washed with 1 *N* HCl and 5% $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated. When mixtures of acylation isomers were produced, the residues were chromatographed on neutral alumina or Florisil eluting with benzene–ethyl acetate mixtures. Otherwise they were directly recrystallized from benzene or ethanol.

Saponification of the Methyl or Ethyl β-Arylamidoacrylates, II. The β-amido esters II were finely ground and suspended in 2-propanol, 200 ml/0.1 mol. A solution of 1.5 equiv of 15 *N* KOH was added with stirring over a 90-min period. The temperature was maintained at 20°. After 16 hr, most of the 2-propanol was removed under reduced pressure and the concentrate diluted with 4 vol of H_2O. This was washed twice with ether and then carefully acidified with dilute HCl. The resulting β-arylamidoacrylic acids were usually recrystallized from MeOH or benzene.

β-Amino-3-bromocrotonophenone (69). A solution of 60 g (0.38 mol) of *tert*-butyl β-aminocrotonate in 500 ml of dry THF was cooled to 0° and combined with 38 g (0.38 mol) of 2,6-lutidine and 82 g (0.38 mol) of 3-bromobenzoyl chloride. The temperature was held at 0° for 5 hr and then allowed to come to room temperature overnight. Work-up and chromatography as described above gave 26 g of the C-acylated ester, *tert*-butyl 3-amino-α-(3-bromobenzoyl)crotonate (**68**), as white platelets from MeOH: mp 89°. Anal. ($C_{15}H_{18}BrNO_3$) C, H, N.

A solution of 26 g (0.076 mol) of this ester in 500 ml of CH_2Cl_2 was cooled to −10° and saturated with HCl gas. After 4 hr the reaction was allowed to warm to 10° and stand overnight. The solution was then washed with H_2O and 5% $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated. Recrystallization from ether–pentane gave 13 g (72%) of the crotonophenone **69** as fine white plates: mp 119°. Anal. ($C_{10}H_{10}BrNO$) C, H, N.

Ethyl β-Amino-α-(*N*-phenylcarboxamido)crotonate (70) and Ethyl β-Phenylureidocrotonate (71). A solution of 29 g (0.23 mol) of ethyl β-aminocrotonate and 27 g (0.23 mol) of phenyl isocyanate in 200 ml of ether was refluxed for 1 hr. When cool the ether was evaporated and the solid residue digested with 400 ml of pentane. The pentane-insoluble material was twice recrystallized from benzene–pentane to give 11 g of the C-acylated isomer as fine white needles: mp 125°. Anal. ($C_{13}H_{16}N_2O_3$) C, H, N. The pentane-soluble isomer was twice recrystallized from pentane to give 9 g of the N-acylated isomer **71** as fine white needles: mp 97°. Anal. ($C_{13}H_{16}N_2O_3$) C, H, N.

***N,N*-Diethyl-β-benzamidocrotonamides, VI.** These were made in about 50% yields by the reaction of the appropriate acid chloride with commercially available *N,N*-diethyl-3-aminocrotonamide in the same manner as that used for the amino esters I.

References

(1) C. K. Cain, *Annu. Rep. Med. Chem.*, 225 (1970).
(2) D. J. Drain, M. J. Daly, B. Davy, M. Horlington, J. G. B. Howes, J. M. Scrunton, and R. A. Selway, *J. Pharm. Pharmacol.*, **22**, 684 (1970).
(3) J. G. Lombardino and E. H. Wiseman, *J. Med. Chem.*, **15**, 848 (1972).
(4) T. Y. Shen, *Top. Med. Chem.*, **1**, 53 (1967).
(5) (a) P. F. Pascoe, *Justus Liebigs Ann. Chem.*, **705**, 109 (1967); (b) G. F. Beattie, J. M. Pryce, and P. J. Taylor, *Chem. Commun.*, 793 (1971).

(6) C. C. Barker, *J. Chem. Soc.*, 317 (1954).
(7) R. L. Augustine, R. F. Bellina, and A. J. Gustavsen, *J. Org. Chem.*, **33**, 1287 (1968).
(8) C. N. R. Rao, "Chemical Applications of Infrared Spectroscopy", Academic Press, New York, N.Y., 1963, p 245.
(9) D. L. Ostercamp, *J. Org. Chem.*, **35**, 1632 (1970).
(10) D. A. Cornforth, A. E. Opara, and G. Read, *J. Chem. Soc.*, 2799 (1969).
(11) R. N. Lacey, *J. Chem. Soc.*, 839 (1954).
(12) (a) L. F. Sancilio, *Proc. Soc. Exp. Biol. Med.*, **127**, 597 (1968); (b) L. F. Sancilio, *J. Pharmacol. Exp. Ther.*, **188**, 199 (1969).
(13) R. Littell and G. R. Allen, Jr., U.S. Patent 3,449,363.

Synthesis and Thin-Layer Chromatographic, Ultraviolet, and Mass Spectral Properties of the Anticoagulant Phenprocoumon and Its Monohydroxylated Derivatives†

Lance R. Pohl,

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122

Rodney Haddock, William A. Garland, and William F. Trager*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of Washington, Seattle, Washington 98195.
Received April 8, 1974

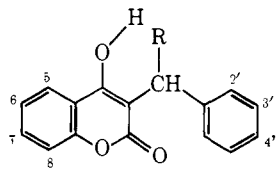
Phenprocoumon and all of its aromatic monohydroxylated derivatives have been synthesized and analyzed by TLC, uv, and chemical ionization mass spectroscopy. By utilization of various combinations of these analytical techniques all of the titled compounds can be uniquely identified.

As a continuation of our studies on the biotransformation of warfarin (1)[1] and the relationship between drug-induced interactions and its metabolism,[1d,2,3] we have begun a study of the metabolism of the closely related oral anticoagulant phenprocoumon (2).

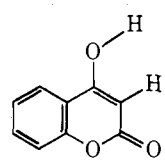
Although the two drugs are structurally quite similar, significant differences in their pharmacologic properties exist. For example, in an extensive clinical study phenprocoumon has been reported to elicit a more stable and reliable hypoprothrombinemic response compared to warfarin.[4] In addition, it is significantly more active than warfarin and has a much longer biologic half-life.[5] The reasons for these differences are at present obscure.

Since warfarin is known to be monohydroxylated in the 6, 7, 8, and 4′ positions in the rat[2,6] and in the 6 and 7 positions in man,[1,7,8] it seemed reasonable to anticipate that phenprocoumon would also be susceptible to aromatic hydroxylation. Indeed, preliminary work (mass spectrometry) in our laboratory had indicated the presence of such species in the urine of rats who had been injected with the drug. Hence, in order to facilitate the unambiguous identification of potential metabolites and to provide standards we embarked upon a synthetic program concurrent with our metabolic studies to characterize all the possible aromatic monohydroxylated derivatives of phenprocoumon.

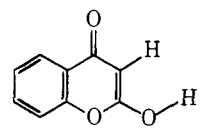
Synthesis. Phenprocoumon (2) can be synthesized by alkylating 4-hydroxycoumarin (3) with 1-phenyl-1-propanol using HCl[9,10] or H_2SO_4[11] as catalysts or by using $POCl_3$[12] as a condensing agent. Alternatively, it can be synthesized by direct alkylation with 1-phenyl-1-bromopropane.[13] As a consequence, these reaction schemes were initially explored for the synthesis of 6-hydroxyphenprocoumon from 4,6-dihydroxycoumarin but were unsuccessful as only intractable tars were obtained. To circumvent the problem of O- vs. C-alkylation in the condensation reaction the 5-, 6-, 7-, and 8-hydroxy positions were initially protected with benzyl groups which were subsequently removed by hydrogenolysis.



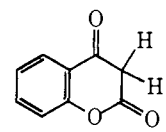
1, R = CH_2COCH_3
2, R = CH_2CH_3



3



4



5

A method similar to that of Hermodson, Barker, and Link[14] was utilized for the synthesis of 5-, 6-, 7-, and 8-benzyloxy-4-hydroxycoumarin and involved the selective‡ monobenzylation of the isomeric dihydroxyacetophenones. The acetophenones were then allowed to react with diethyl carbonate by the method of Dickenson[16] to yield the benzyloxy-4-hydroxycoumarins. Condensation of these materials with 1-phenylpropanol or 1-phenyl-1-bromopropane also led to intractable tars.

Since coumarin 3 can exist in two other tautomeric forms,[17] the chromone structure 4 and the diketo structure 5, it seemed that alkylation of 4-hydroxycoumarin§ and its benzyloxy derivatives might be possible using conditions that are commonly employed for the alkylation of β-keto esters.[18] Sodium acetate was chosen as the base, since it was sufficiently strong to abstract the 4-hydroxy proton[19] but also weak enough to prevent significant dehydrobro-

†This investigation was supported in part by NIH Training Grant No. 5-TO1-GM 00728, by a Washington State Heart Association Grant, and by an American Foundation for Pharmaceutical Education Fellowship Grant. Funds for the modification of the MS-9 to permit chemical ionization were provided by a grant from the Seattle Foundation.

‡The ^1H NMR absorption of the intramolecular hydrogen-bonded ortho groups[15] at approximately 11 ppm clearly indicated the selectivity of benzylation.

§For a review of the reactions of 4-hydroxycoumarin, see Zanten and Nauta.[18]