(8) A. Lespagnol, M. Debaert, J. Mizon, and C. Mizon-Capron, *Therapie*, **25**, 707 (1970); *Chem. Abstr.*, **74**, 19728t (1971).
(9) R. G. Jones, *J. Amer. Chem. Soc.*, **71**, 644 (1949).
(10) I. E. Balaban, *J. Chem. Soc.*, 2423 (1932).
(11) R. Weidenhagen and H. Wegner, *Ber.*, **70**, 2309 (1937).
(12) G. S. Reddy, L. Mandell, and J. H. Goldstein, *J. Chem. Soc.*, 1414 (1963).
(13) F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).
(14) C. Musante and P. Pino, *Gazz. Chim. Ital.*, **77**, 199 (1947).
(15) W. G. Forsyth and F. L. Pymann, *J. Chem. Soc.*, **127**, 573 (1925).
(16) L. P. Kuhn, *J. Amer. Chem. Soc.*, **73**, 1510 (1951).
(17) L. I. Krimen, *Org. Syn.*, **50**, 1 (1970).
(18) I. E. Balaban, *J. Chem. Soc.*, 268 (1930).
(19) G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1648 (1959).
(20) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, Wiley, New York, N. Y., 1967, p 20.
(21) G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 2937 (1962).
(22) M. Conrad and A. Schulz, *Ber.*, **42**, 735 (1909).
(23) H. Auterhoff and M. F. Hebler, *Arzneim.-Forsch.*, **9**, 621 (1959).
(24) G. P. Hager, J. C. Krantz, Jr., Dranta, Jr., and J. B. Harman, *J. Amer. Pharm. Ass.*, **42**, 36 (1953).
(25) R. M. Hoskinson, *Aust. J. Chem.*, **21**, 1913 (1968).
(26) T. H. Sidall and R. H. Garner, *Tetrahedron Lett.*, 3512 (1966).
(27) H. A. Staab and D. Lauer, *Tetrahedron Lett.*, 4593 (1966).
(28) J. N. Wells, C. E. Baird, and J. G. Hardman, Abstracts of Federal Proceedings, Atlantic City, N. J., April 1974.
(29) J. A. Beavo, J. G. Hardman, and E. W. Sutherland, *J. Biol. Chem.*, **245**, 5649 (1970).
(30) C. Cosar, C. Crisan, R. Horclois, R. M. Jacqb, J. Robert, G. Tchelitcheff, and R. Vaupre, *Arzneim.-Forsch.*, **16**, 23 (1966).
(31) F. Johnson and W. A. Nasutavicus, *J. Org. Chem.*, **29**, 153 (1964).

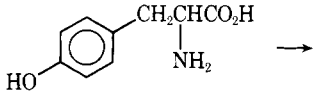
Inhibition of Tyrosinase-Catalyzed Melanin Formation by Catechol Phenyl Sulfones

Jerome F. Siuda*,† and Ayman Habal

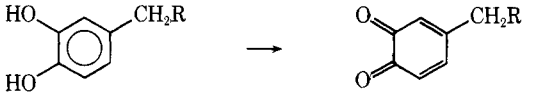
Department of Medicinal Chemistry, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261.
Received March 14, 1974

Catechol phenyl sulfones were synthesized and evaluated as potential inhibitors of tyrosinase-mediated Dopa melanin formation. The results suggest that the dopamine analogs, 2,3-dihydroxy-5-(β-aminoethyl)diphenyl sulfone hydrochloride (**9b**) and 2,3-dihydroxy-5-(β-aminoethyl)-4′-aminodiphenyl sulfone dihydrochloride (**9d**) as well as the Dopa analogs 2,3-dihydroxy-5-(β-alanyl)diphenyl sulfone hydrochloride (**10b**) and 2,3-dihydroxy-5-(β-alanyl)-4′-aminodiphenyl sulfone dihydrochloride (**10d**), are noncompetitive inhibitors of enzyme-catalyzed melanin formation. The methylcatechol analogs, 2,3-dihydroxy-5-methyldiphenyl sulfone (**8a**) and 2,3-dihydroxy-5-methyl-4′-aminodiphenyl sulfone (**8c**), had no effect on the formation of melanin, indicating that the β-aminoethyl side chain was required for inhibitory activity in this series of compounds. Although **10d** was the most effective inhibitor of melanin formation, it did not inhibit the tyrosinase-catalyzed conversion of tyrosine to Dopa.

Tyrosinase (E.C. 1.10.3.1) is an enzyme capable of performing a dual function; it catalyzes the hydroxylation of tyrosine (1) to 3,4-dihydroxyphenylalanine (**4b**, Dopa) and subsequently oxidizes the catechol to a labile *o*-quinone **7b**.[1,2] The enzyme also acts on a variety of other catechols converting them to quinones. The reactive quinones may



1

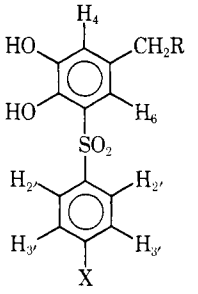


2, R = H
3a, R = CH_2NHCbz
b, R = CH_2NH_2
4a, R = $CH(NHCbz)CO_2CH_3$
b, R = $CH(NH_2)CO_2H$

5, R = H
6a, R = CH_2NHCbz
b, R = CH_2NH_2
7a, R = $CH(NHCbz)CO_2CH_3$
b, R = $CH(NH_2)CO_2H$

undergo a number of reactions including ring cyclization, addition with nucleophiles, and ring cleavage. These varied reactions usually result in the production of melanin pigments which are heterogeneous polymers. Primarily through the efforts of Raper[3,4] and Mason[5] and their coworkers, a scheme of melanogenesis has been proposed

†Dedicated to my friend and former research professor, Dr. Alfred Burger.



8a, R = H; X = H
b, R = H; X = NHAc
c, R = H; X = NH_2
9a, R = CH_2NHCbz; X = H
b, R = $CH_2\overset{+}{N}H_3Cl^-$; X = H
c, R = CH_2NHCbz; X = NHAc
d, R = $CH_2\overset{+}{N}H_3Cl^-$; X = $\overset{+}{N}H_3Cl^-$
10a, R = $CH(NHCbz)CO_2CH_3$
b, R = $CH(\overset{+}{N}H_3Cl^-)CO_2H$; X = H
c, R = $CH(NHCbz)CO_2CH_3$; X = NHAc
d, R = $CH(\overset{+}{N}H_3Cl^-)CO_2H$; X = $\overset{+}{N}H_3Cl^-$

whereby melanin is formed from Dopa proceeding through quinoidal and indolic intermediates. In the cancer, melanoblastoma (malignant melanoma), an abnormal amount of melanin is usually produced mediated by tyrosinase in an active state.[6] Unfortunately, chemotherapeutic agents

Table I. Catechol Phenyl Sulfones

Compd	Mp, °C (solvent)	Yield, %	Formula	Analyses[f]
8a	139–141 (CH_2Cl_2–pet. ether)	93[c]	$C_{13}H_{12}O_4S$	C, H
8b	260–260.5 ($CHCl_3$–MeOH)	65[c]	$C_{15}H_{15}NO_5S$	C, H, N
8c	245–246.5 (12 *N* HCl)	95[d,e]	$C_{13}H_{13}NO_4S$	C, H, N
9a	170.5–171.5 ($CHCl_3$–Et_2O)	74[c]	$C_{22}H_{21}NO_6S$	C, H, N
9b	259–261 (12 *N* HCl)	99[d]	$C_{14}H_{16}ClNO_4S$	C, H, N
9c	189–190 ($CHCl_3$–MeOH)	67[c]	$C_{24}H_{24}N_2O_7S$	C, H, N
9d	263 dec (12 *N* HCl)	98[d]	$C_{14}H_{18}Cl_2N_2O_4S$	C, H, N
10a	149.5–151.5[a]	79[c]	$C_{24}H_{23}NO_8S$	C, H, N
10b	221.5 dec (12 *N* HCl)	96[d]	$C_{15}H_{16}ClNO_6S$	C, H, N
10c	186–187[b] ($EtOH-H_2O$)	71[c]	$C_{26}H_{26}N_2O_9S\cdot H_2O$	C, H, N
10d	191 dec (12 *N* HCl)	99[d]	$C_{15}H_{18}Cl_2N_2O_6S$	C, H, N

[a] Obtained by trituration with ether. [b] From chromatography on silicic acid. [c] Calculated from catechol reactants. [d] Calculated from protected catechol phenyl sulfone reactants. [e] Yield of free amine. [f] Elemental analyses performed by Spang Microanalytical Labs, Ann Arbor, Mich., and Galbraith Laboratories, Inc., Knoxville, Tenn.

in the treatment of melanoblastoma have not given a predictable and favorable response.[7,8] Inhibitors of tyrosinase may be useful as antitumor substances and/or as an aid in understanding the biochemical etiology of the disease.

The synthesis of catechol phenyl sulfones **8–10** was undertaken for a number of reasons. Catecholamine-type compounds are intermediates in melanin formation[9] and synthetic analogs are potential inhibitors of catechol oxidation.[10] The addition of aromatic sulfinic acids to quinones is rapid[11] and essentially quantitative, thus providing a possible means of trapping labile quinones formed *in situ* or in physiological systems. Furthermore, certain hydroxy- and amino-substituted diphenyl sulfones possess antibacterial[12] and antileprotic[13,14] activity.

Chemistry. The catechol phenyl sulfones **8a,c, 9b,d,** and **10b,d** were prepared by the addition of an aromatic sulfinic acid **11** to a quinone generated *in situ*, followed by hydrolysis with HCl. Solutions of the quinones were first obtained by oxidizing the corresponding catechol with silver oxide[15,16] in an anhydrous organic solvent. After fil-

5. **6a**, or **7a** + *p*-$XC_6H_4SO_2H$ ⟶

11a, X = H

b, X = NHAc

8a,b, **9a,c**, or **10a,c** $\xrightarrow{HCl}$ **8c**, **9b,d**, or **10b,d**

tration of the silver oxide, the solution of the quinone was added to a solution containing an excess of the sulfinic acid which caused the immediate decolorization of the red quinone solution. With benzenesulfinic acid (**11a**) as the nucleophile, the reactions were conducted in nonalcoholic solutions and unreacted acid was removed by washing the organic phase with dilute $NaHCO_3$. However, *p*-acetamidobenzenesulfinic acid **11b** was only soluble in methanol or ethanol and the work-up was carried out in a different fashion. Evaporation of the sulfone solutions to dryness and sonification of the residue with chloroform or ethyl acetate enabled the product to be taken up in the solvent, leaving behind excess **11b** which was removed by filtration.

The amino group of dopamine (**3b**) and both the amino and carboxyl functions of Dopa (**4b**) had to be protected to avoid ring cyclization[4,17] during the oxidation of the catechols to the corresponding quinones. Removal of the protective groups in **8b, 9a,c,** and **10a,c** was easily accomplished by refluxing with HCl. In so doing, the monohydrochlorides **9b** and **10b** and the dihydrochlorides **9d** and **10d** were obtained as stable salts at 110° and 0.1 mm. Interestingly, the hydrolysis of **8b** with 3 *N* HCl gave in large part the free amine **8c**. Only small amounts of the hydrochloride of **8c** could be recovered and this substance readily lost HCl to yield the free amine. Nmr observation of the free amine and its hydrochloride demonstrated the interconversion of the two compounds.

A number of sites on the quinone nucleus are available for nucleophilic addition. However, spectroscopic data clearly indicated that attack of the sulfinic acids proceeded in a manner to give the substitution products illustrated. Using **8a** and **8b** as models, nmr studies revealed the presence of two doublets (J = 3 Hz) near δ 6.9 and 7.1, representing the meta splitting[18] of the H-4 and H-6 protons. This same pattern of doublets was observed in the other products, the chemical shift dependent upon the solvent utilized. In addition to the nmr observations, most of the compounds clearly revealed the presence of a sharp band near 840 cm^{-1} indicating 1, 2, 3, 5 aromatic substitution.[19] The attack of the sulfinic acids represents a 1,6 addition at the least sterically hindered position of the quinone. This same type of addition for similar quinones has been observed with other types of nucleophilic agents.[20,21]

Table I lists the catechol phenyl sulfones synthesized. Determination of purity for each of the compounds was performed in the usual manner, including tlc in several different solvent systems. All compounds gave one spot on tlc analysis. In addition, compounds **8b, 9a,c,** and **10a,c** were not tested for enzyme inhibition properties due to their low solubility in most solvents.

Enzyme Inhibition. The sulfones **8a,c, 9b,d,** and **10b,d** were used to study, *in vitro*, the inhibition of Dopa melanin formation catalyzed by mushroom tyrosinase (for details, see Experimental Section). The enzymatic reaction was found to be linear with respect to substrate concentration. K_m and V_{max} were calculated to be 8.41×10^{-3} and 0.89, respectively. Figure 1 illustrates that melanin formation is inhibited by the catecholamine analogs **9b,d** and **10b,d** but not by the 4-methylcatechol analogs, **8a,c**. At 3 min, the per cent inhibition for **9b,d** and **10b,d** at 4×10^{-3} *M* was 45, 54, 73, and 79%, respectively. Because of the limited solubility of the inhibitors, concentrations greater than 4×10^{-3} *M* were not evaluated. Lineweaver–Burk[22] plots obtained from a limited number points suggested that the catecholamine analogs inhibit Dopa melanin formation by a noncompetitive mechanism. No changes were observed when the tested analogs were incu-

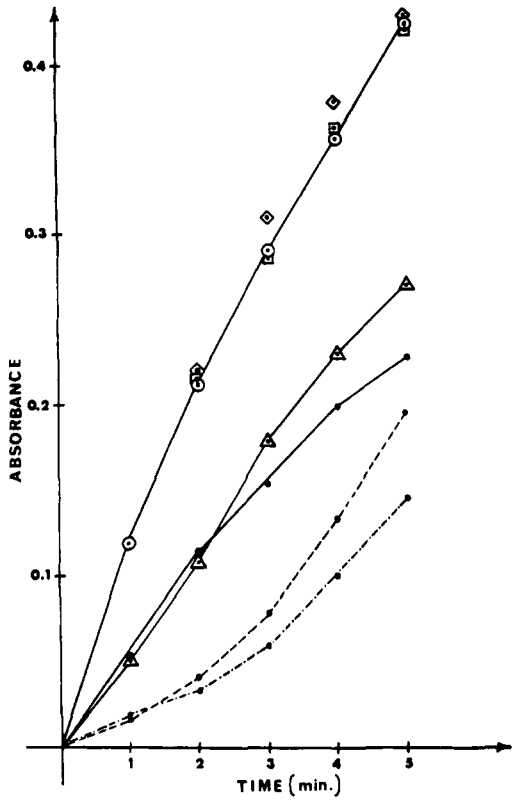


Figure 1. Inhibition of Dopa melanin formation by tyrosinase using 4×10^{-3} *M* Dopa and inhibitors followed at 475 nm: ⊙, no inhibitor added; ⊡, **8a**; ◈, **8c**; ▲, **9b**; ●—●, **9d**; ●- - -●, **10b**; ●···●, **10d**.

bated with tyrosinase in the absence of Dopa, demonstrating that the sulfones were not substrates.

To determine the specificity of the inhibition, the most effective inhibitor **10d** was evaluated for its ability to hinder the conversion of tyrosine (**1**) to Dopa (**4b**). From Figure 2, it may be seen that **10d** had no effect on the hydroxylation of **1** to **4b**; however, the formation of melanin was obviated.

Discussion

In this study as well as others,[23,24] the appearance of absorption at 475 nm is related to the degree of melanin formation. Although some of the compounds inhibit Dopa melanin formation and apparently do not inhibit the hydroxylation of **1** go **4b** (cresolase activity[2]), it is not known whether the evaluated substances inhibit the oxidation of **4b** to the quinone **7b** (catecholase activity[2]). This latter assay could not be determined accurately since the uv absorption of the inhibitors interferes with the measurement of the absorption of Dopa quinone at 305–310 nm.[23,25,26]

From the results obtained, replacement of a hydrogen atom at C-5 of the catechols **2–4** by a phenylsulfonyl group may sufficiently alter the oxidation potential of the catechols, causing a drastic change in the biochemical properties of these substances. Although the methylcatechol analogs did not inhibit melanogenesis, these compounds did not appear to be substrates for oxidation under the conditions of the experiment. In the case of analogs resembling dopamine and Dopa, there was significant inhibition of melanin formation. The results indicate the β-aminoethyl side chain plays a role during the inhibition process. Due to the apparent noncompetitive nature of the inhibitors, the side chain may be binding to the enzyme at a site other than the active site(s). Another possibility is that during enzymic oxidation, Dopa quinone (**7b**)

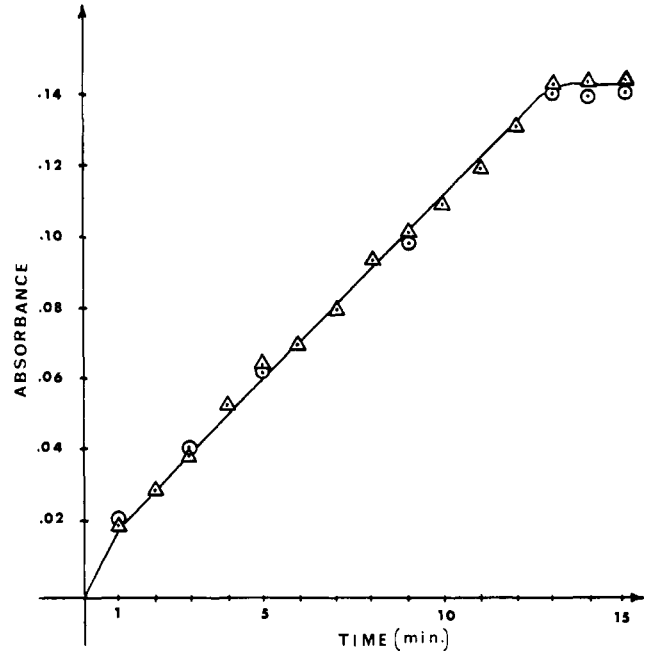


Figure 2. Enzymatic oxidation of L-tyrosine to Dopa followed at 280 nm: ▲, no inhibitor added; ⊙, with 4×10^{-3} *M* **10d** added.

is indeed formed, but immediately reacts intermolecularly with the nucleophilic portion of the aminoethyl chain[23] thus affecting the production of melanin. On the other hand, if this was the situation, it might be expected that the dopamine analogs **9b** and **9d** would be more efficient inhibitors due to the expected greater nucleophilicity of the amino group relative to the amino group of Dopa analogs **10b** and **10d**. These latter substances under the conditions of the experiment are believed to be in the zwitterionic form. Thus, the positively charged aliphatic nitrogen would be less likely to attack the quinone as a nucleophilic agent. The fact that the Dopa analogs are better inhibitors than the dopamine-like compounds is an indication that the side-chain amine may not react with the generated quinones.

Experimental Section

Melting points were determined using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Ir data were obtained with a Perkin-Elmer Model 267 using KBr pellets. Nmr spectra were recorded on a Varian A-60 instrument. Spectra in organic solvents were obtained with TMS as a reference, whereas in D_2O the internal standard was DSS. Benzenesulfinic acid was prepared by acidification of its sodium salt. *p*-Acetamidobenzenesulfinic acid was obtained by the reduction of the sulfonyl chloride.[27] Silver oxide was freshly prepared.

Mushroom tyrosinase was obtained from Nutritional Biochemicals Co., Cleveland, Ohio. Enzyme inhibition studies were conducted at 25° in phosphate buffer (pH 6.8) similar to literature procedures.[23,24] As a control, Dopa was added to the tyrosinase and the oxidation was followed at 475 nm using a Bausch and Lomb Spectronic 20 spectrophotometer. During the inhibition studies, inhibitor was first added to the enzyme followed immediately by the addition of Dopa. Readings were taken at 1-min intervals over a 5-min period. Each point on the plot represents an average of three trials. The formation of Dopa from tyrosine was followed at 280 nm.[23,28]

2,3-Dihydroxy-5-methyldiphenyl Sulfone (8a). A solution of freshly prepared quinone **5**[29] (2.7 g, 0.022 mol) in 30 ml of $CHCl_3$ was added to a solution of **11a** in anhydrous ether (3.14 g, 0.022 mol) and the red quinone solution was immediately decolorized. The solution was evaporated to give 5.4 g (93%) of a yellow solid which after several recrystallizations from CH_2Cl_2–petroleum ether provided a white crystalline solid of analytical purity: mp 139–141°; ir 1280, 1140, and 842 cm^{-1}; nmr (DMSO-d_6) δ 2.23 (s, 3 H, Ar CH_3), 6.9 (d, J = 3 Hz, 1 H, Ar H-4 or H-6), 7.2 (d, J = 3 Hz, 1 H, Ar H-4

or H-6), 7.5–8.0 (m, 5 H, Ar H-2′, H-3′, H-4′). *Anal.* ($C_{13}H_{12}O_4S$).

2,3-Dihydroxy-5-methyl-4′-acetamidodiphenyl Sulfone (8b). A solution of **2** (6.0 g, 0.048 mol) in 60 ml of anhydrous Et_2O was added to a well-stirred suspension of silver oxide (22.6 g, 0.096 mol) and 30 g of anhydrous Na_2SO_4 in 200 ml of Et_2O. After stirring for 3 min, the red quinone mixture was filtered into a stirred solution of **11b** (9.65 g, 0.049 mol) in 50 ml of anhydrous MeOH. The solution was evaporated to dryness and the residue washed with 3 × 50 ml of Et_2O. To the solid was then added 300 ml of hot $CHCl_3$ to dissolve the sulfone and the unreacted sulfinic acid was removed by filtration. Evaporation of the $CHCl_3$ and several recrystallizations from $CHCl_3$–MeOH afforded 7.3 g (65%) of a white solid: mp 260–260.5°; ir 1695, 1280, 1125, and 835 cm^{-1}; nmr (DMSO-d_6) δ 2.10 (s, 3 H, CH_3CON), 2.23 (s, 3 H, Ar CH_3), 6.92 (d, J = 3 Hz, 1 H, Ar H), 7.15 (d, J = 3 Hz, 1 H, Ar H), 7.78 (s, 4 H, Ar H′), 9.25, 9.85, and 10.25 (each br, 1 H each, OH and NH). *Anal.* ($C_{15}H_{15}NO_5S$).

2,3-Dihydroxy-5-methyl-4′-aminodiphenyl Sulfone (8c). A suspension of **8b** (6.00 g, 0.0187 mol) in 150 ml of 3 *N* HCl was refluxed for 6 hr and the clear solution cooled to room temperature. The crystalline solid that formed was filtered and dried, giving 4.95 g (95%) of **8c**: mp 245–246.5°; ir 1280, 1124, and 844 cm^{-1}; nmr (DMSO-d_6) δ 2.18 (s, 3 H, Ar CH_3), 6.58 (d, J = 9 Hz, 2 H, Ar H-3′), 6.79 (d, J = 3 Hz, 1 H, Ar H-4 or H-6), 7.08 (d, J = 3 Hz, 1 H, Ar H-4 or H-6), 7.55 (d, J = 9 Hz, 2 H, Ar H-2′). *Anal.* ($C_{13}H_{13}NO_4S$).

After filtration of the free amine from the above hydrolysis, the filtrate was concentrated to 20 ml and 0.1 g of a white crystalline solid appeared: mp 244° dec; ir 2600, 2520, 1290, 1132, and 842 cm^{-1}; nmr (DMSO-d_6) δ 2.19 (s, 3 H, Ar CH_3), 6.90 (d, J = 3 Hz, 1 H, Ar H-4 or H-6), 7.0–7.25 (m, 3 H, Ar H-4 or H-6 + Ar H-3′), 7.75 (d, J = 9 Hz, 2 H, Ar H-2′), 9.25 (s, 5 H, OH + $^+NH_3$). Addition of NaOD resulted in the loss of the singlet at δ 9.25 (exchangeable protons) and the resolution of the multiplet to give a spectrum almost identical with that of the free amine. Similarly, addition of DCl to the amine **8c** caused the Ar H-3′ protons to shift *ca.* δ 0.5 downfield, *i.e.*, from δ 6.58 to *ca.* 7.0–7.2. The spectroscopic data indicated the substance to be the hydrochloride of **8c**. This substance readily lost HCl to form **8c**.

2,3-Dihydroxy-5-(*N*-carbobenzyloxy-β-aminoethyl)diphenyl Sulfone (9a). To a well-stirred solution of **3a**[16] (5.0 g, 0.0129 mol) in 300 ml of $CHCl_3$ and 5 ml of MeOH was added 10 g of anhydrous Na_2SO_4 and 16.1 g (0.0516 mol) of silver oxide. The mixture was stirred vigorously for 10 min at room temperature and the red solution filtered into a stirred solution of **11a** (3.09 g, 0.021 mol) in 50 ml of Et_2O. The pale yellow sulfone solution was evaporated to dryness and the yellow residue digested with 10 ml of Et_2O. A white solid formed which was filtered and recrystallized from $CHCl_3$–Et_2O to give 5.45 g (74%) of a white solid: mp 170.5–171.5°; ir 1710, 1285, 1138, and 845 cm^{-1}; nmr (DMSO-d_6) δ 2.6–2.8 (m, 2 H, Ar CH_2C), 3.0–3.4 (m, 2 H, CH_2N), 5.02 (s, 2 H, Ar CH_2O–), 6.92 (d, J = 3 Hz, 1 H, Ar H), 7.25 (d, J = 3 Hz, 1 H, Ar H), 7.30 [s, 5 H, $C_6H_5OC(=O)$–], 7.4–8.0 (m, 5 H, Ar H′). *Anal.* ($C_{22}H_{21}NO_6S$).

2,3-Dihydroxy-5-β-aminoethyldiphenyl Sulfone Hydrochloride (9b). A suspension of **9a** (5.4 g, 0.0124 mol) in 100 ml of 3 *N* HCl plus 20 ml of EtOH was refluxed for 40 hr. The clear solution was evaporated to dryness and the solid washed with 2 × 50 ml of Et_2O. Recrystallization of the solid from 12 *N* HCl gave 4.2 g (99%) of a fine white solid: mp 259–261°; ir 2730, 2550, 1280, and 1145 cm^{-1}; nmr (DMSO-d_6) δ 2.7–3.3 (br, 4 H, $-CH_2CH_2-$), 7.1 (d, J = 2 Hz, 1 H, Ar H), 7.3 (d, J = 2 Hz, 1 H, Ar H), 7.4–8.1 (m, 5 H, Ar H′), 8.1–8.4 (m, 2 H, OH? disappear in D_2O). *Anal.* ($C_{14}H_{16}ClNO_4S$).

2,3-Dihydroxy-5-(*N*-carbobenzyloxy-β-aminoethyl-4′-acetamido)diphenyl Sulfone (9c). A solution of the quinone **6a** was prepared from 5.0 g (0.0129 mol) of **3a** as described above in the synthesis of **9a**. The quinone mixture was filtered into a stirred solution of **11b** (4.16 g, 0.021 mol) in 100 ml of MeOH. The solution was evaporated to dryness and the resulting solid digested with 150 ml of $CHCl_3$ leaving behind unreacted **11b**. The $CHCl_3$ solution of **9c** was concentrated to 25 ml and 50 ml of Et_2O was added to give an oil. This mixture was then refluxed until the oil solidified. The solid was recrystallized several times from $CHCl_3$–MeOH giving 5.6 g (67%) of a white solid: mp 189–190°; ir 1708, 1696, 1680, 1282, 1130, and 838 cm^{-1}; nmr (DMSO-d_6) δ 2.12 [s, 3 H, $CH_3C(=O)N$], 2.6–3.4 (m, 4 H, $-CH_2CH_2-$), 5.05 (s, 2 H, Ar CH_2O–), 6.92 (d, J = 2.5 Hz, 1 H, Ar H), 7.25 (d, J = 3 Hz, 1 H, Ar H), 7.31 (s, 4 H, Ar H′). *Anal.* ($C_{24}H_{24}N_2O_7S$).

2,3-Dihydroxy-5-β-aminoethyl-4′-aminodiphenyl Sulfone Dihydrochloride (9d). **9c** (5.2 g, 0.0108 mol) was hydrolyzed to **9d** as described above in the preparation of **9b**. Recrystallizations from 12 *N* HCl gave 4.0 g (98%) of a fluffy white solid: mp 263° dec; ir 2720, 2550, 1280, 1128, and 865 cm^{-1}; nmr (D_2O) δ 2.7–3.3 (m, 4 H, $-CH_2CH_2-$), 7.02 (d, J = 3 Hz, 1 H, Ar H), 7.28 (d, J = 3 Hz, 1 H, Ar H), 7.42 (d, J = 9 Hz, 2 H, Ar H-3′), 7.91 (d, J = 9 Hz, 2 H, Ar H-2′). *Anal.* ($C_{14}H_{18}Cl_2N_2O_4S$).

Methyl 3,4-Dihydroxy(*N*-carbobenzyloxy)-β-phenylalanate (4a). A solution of *N*-carbobenzyloxydihydroxyphenylalanine[17] (16.0 g, 0.048 mol) in 50 ml of absolute MeOH was heated at 50° while bubbling gaseous HCl into the mixture. The solution was evaporated to dryness, giving 16.5 g (99%) of the methyl ester: mp 116–118°; ir 3515, 3480, 1732, and 1690 cm^{-1}; nmr (DMSO-d_6) δ 2.6–2.9 (m, 2 H, Ar CH_2C), 3.62 (s, 3 H, OCH_3), 3.9–4.4 (m, 1 H, CH), 5.05 (s, 2 H, Ar CH_2O), 6.3–6.9 (m, 3 H, Ar H), 7.25 (s, 5 H, Ar H), 8.3–8.8 (br, 2 H, OH, disappear in D_2O).

Methyl 3,4-Dihydroxy-5-phenylsulfonyl(*N*-carbobenzyloxy)-β-phenylalanate (10a). To a solution of **4a** (5.20 g, 0.015 mol) in 200 ml of $CHCl_3$ was added 20 g of anhydrous Na_2SO_4 and 14.9 g (0.06 mol) of silver oxide. The mixture was vigorously stirred at 15–20° for 25 min. The quinone solution was filtered into a stirred solution of **11a** (3.5 g, 0.02 mol) in 100 ml of Et_2O. After addition was completed, the solution was washed successively with 100 ml of cold 5% $NaHCO_3$, 100 ml of 10% HCl, and finally H_2O. The organic phase was dried ($MgSO_4$) and evaporated to dryness furnishing an oil. Upon sonification with 50 ml of Et_2O, 5.79 g (79%) of white solid formed: mp 149.5–151.5°; ir 1770, 1680, 1280, and 1150 cm^{-1}; nmr (DMSO-d_6) δ 2.8–3.6 (m, 3 H, CH_2CH), 3.65 (s, 3 H, OCH_3), 5.05 (s, 2 H, Ar CH_2O–), 7.00 (d, J = 3 Hz, 1 H, Ar H), 7.32 (s, 5 H, benzyloxy C_6H_5), 7.5–8.1 (m, 6 H, Ar H, and $SO_2C_6H_5$). *Anal.* ($C_{24}H_{23}NO_8S$).

2,3-Dihydroxy-5-β-alanyldiphenyl Sulfone Hydrochloride (10b). A suspension of **10a** (4.13 g, 0.0085 mol) was refluxed for 6 hr with 100 ml of 3 *N* HCl and 10 ml of EtOH. Similar to the procedures described above, the product obtained was recrystallized several times from 12 *N* HCl giving 3.0 g (96%) of a fine white powder: mp 221.5° dec; ir 2510, 1738, 1285, 1145, and 874 cm^{-1}; nmr (D_2O) δ 2.90 (d, J = 7 Hz, 2 H, Ar CH_2), 4.18 (t, J = 7 Hz, 1 H, $CHCO_2$), 6.85 (d, J = 3 Hz, 1 H, Ar H), 7.0–7.7 (m, 6 H, Ar H and $SO_2C_6H_5$). *Anal.* ($C_{15}H_{16}ClNO_6S$).

Methyl 3,4-Dihydroxy-4′-acetamidophenylsulfonyl-5-(*N*-carbobenzyloxy)-β-phenylalanate (10c). A solution of **4a** (5.20 g, 0.015 mol) was oxidized as described above and treated with **11b** (5.5 g, 0.03 mol) in 100 ml of MeOH. The yellow solid obtained after evaporation of the reaction solvent was sonificated in 500 ml of $CHCl_3$ and filtered to remove insoluble **11b**. The $CHCl_3$ solution was then evaporated to 20 ml and chromatographed on silicic acid. Elution with $CHCl_3$–MeOH (7:1) gave 5.8 g (71%) of the sulfone. Recrystallization from EtOH–H_2O furnished a hydrate: mp 186–187°; ir 1755, 1700, 1670, 1235, 1130, and 830 cm^{-1}; nmr (acetone-d_6) δ 2.15 (s, 3 H, CH_3CON), 3.0–3.3 (m, 2 H, Ar CH_2C), 3.65 (s, 3 H, OCH_3), 5.05 (s, 2 H, Ar CH_2O), 7.05 (d, J = 3 Hz, 1 H, Ar H), 7.30 (s, 6 H, benzyloxy C_6H_5 and Ar H), 7.88 (s, 4 H, Ar H′). *Anal.* ($C_{26}H_{26}N_2O_9S \cdot H_2O$).

2,3-Dihydroxy-5-β-alanyl-4′-aminodiphenyl Sulfone Dihydrochloride (10d). Similar to the hydrolysis described above, 5.8 g (0.011 mol) of **10c** afforded a solid which when recrystallized from 12 *N* HCl gave 4.52 g (99%) of a pure crystalline white solid: mp 191° dec; ir 2560, 1730, 1285, and 1140 cm^{-1}; nmr (D_2O) δ 3.45 (d, J = 7 Hz, 2 H, Ar CH_2), 4.65 (m, 1 H, CH), 7.45 (d, J = 3 Hz, 1 H, Ar H), 7.55 (d, J = 3 Hz, 1 H, Ar H), 7.82 (d, J = 8.5 Hz, 2 H, Ar H-3′), 8.22 (d, J = 8.5 Hz, 2 H, Ar H-2′). *Anal.* ($C_{15}H_{18}Cl_2N_2O_6S$).

Acknowledgment. The investigation was supported in part by Grants NIH CA-11959 and American Cancer Society IN-58 I.

References

(1) H. S. Mason, *Advan. Enzymol.*, **19**, 79 (1957).
(2) J. M. Nelson and C. R. Dawson, *Advan. Enzymol.*, **4**, 99 (1944).
(3) H. S. Raper, *Biochem. J.*, **21**, 89 (1927).
(4) W. C. Evans and H. S. Raper, *Biochem. J.*, **31**, 2162 (1937).
(5) H. S. Mason, *J. Biol. Chem.*, **172**, 83 (1948).
(6) M. Seiji and K. Miyazaki, *J. Invest. Dermatol.*, **57** (5), 316 (1971).
(7) F. D. Johnson and E. M. Jacobs, *Cancer*, **27b**, 1306 (1971).

(8) J. H. Moon, *Cancer*, **25 (2)**, 468 (1970).
(9) J. N. Attie and R. A. Khafif in "Melanotic Tumors," Charles C Thomas, Springfield, Ill., 1964, Chapter 1.
(10) J. McGuire and J. Hendnie, *J. Invest. Dermatol.*, **57 (4)**, 256 (1971).
(11) Y. Ogata, Y. Sawaki, and M. Isono, *Tetrahedron*, **26**, 731 (1970).
(12) S. Pickholz, *J. Chem. Soc.*, 685 (1946).
(13) H. H. Fox in "Medicinal Chemistry," 2nd ed, A. Burger, Ed., Interscience, New York, N. Y., 1970.
(14) W. T. Colwell, G. Chen, V. H. Brown, J. I. DeGraw, and J. H. Peters, *J. Med. Chem.*, **17**, 142 (1974).
(15) H. Jackson and T. Koch, *Amer. Chem. J.*, **26**, 31 (1901).
(16) S. Senoh and B. Witkop, *J. Amer. Chem. Soc.*, **81**, 6222 (1959).
(17) A. Kaiser, W. Koch, M. Scheer, and U. Wölcke, *Helv. Chim. Acta*, **53 (7)**, 1708 (1970).
(18) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," Wiley, New York, N. Y., 1964.
(19) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962.
(20) G. Prota, S. Grescenzi, G. Misuraca, and R. A. Nicolaus, *Experientia*, **26 (10)**, 1058 (1970).
(21) H. Sanada, R. Suzue, Y. Nakashima, and S. Kawada, *Biochim. Biophys. Acta*, **261**, 258 (1972).
(22) M. Dixon and E. C. Webb, "Enzymes," Academic Press, New York, N. Y., 1958.
(23) H. S. Mason and E. W. Peterson, *Biochim. Biophys. Acta*, **111**, 134 (1965).
(24) T. Nagatsu, Y. Sudo, T. Okada, H. Umezawa, and T. Takeuchi, *Experientia*, **28 (6)**, 634 (1972).
(25) H. S. Mason, *J. Biol. Chem.*, **168**, 433 (1947).
(26) R. Hartley and J. A. Smith, *Biochem. Pharmacol.*, **21 (15)**, 2007 (1972).
(27) S. Smiles and C. M. Bere, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1941, p 7.
(28) "Worthington Enzyme Manual," Worthington Biochemical Corp., Freehold, N. J., 1972, p 39.
(29) J. Cason, *Org. React.*, **4**, 305 (1948).

Nonsteroidal Antiinflammatory Agents. 1. 2,4-Diphenylthiazole-5-acetic Acid and Related Compounds

Kevan Brown, David P. Cater, John F. Cavalla, David Green, Robert A. Newberry,* and Alan B. Wilson

Wyeth Institute of Medical Research, Taplow, Maidenhead, Berkshire SL6 OPH, England. *Received February 4, 1974*

A variety of novel 2,4-diarylthiazole-5-acetic acids and related compounds was prepared by the Hantzsch thiazole synthesis and evaluated as antiinflammatory agents on the carrageenin-induced edema assay in the rat. Two compounds, namely 4-(4-chlorophenyl)-2-phenylthiazole-5-acetic acid (**24**) and 4-(4-chlorophenyl)-2-(3-methylphenyl)-thiazole-5-acetic acid (**31**), were found to possess activity comparable with indomethacin. Derivatives of the acidic side chain such as esters, amides, hydroxamic acid, β-propionic acids, α-propionic acids, and a tetrazole were all less active than the parent compounds. Compound **24** was five times as effective as phenylbutazone against adjuvant-induced polyarthritis.

In 1963 indomethacin, 1-*p*-chlorobenzoyl-5-methoxy-2-methylindole-3-acetic acid, was reported by Shen, *et al.*,[1] to possess antiinflammatory activity against carrageenin-induced edema in the rat hind paw. This knowledge, together with the fact that aspirin and phenylbutazone (two nonsteroidal antiinflammatory drugs of choice at that time) were both acidic compounds, induced many workers to investigate other aryl- and heteroarylalkanoic acids (for a review, see ref 2).

This paper describes studies based upon the discovery that 2,4-diphenylthiazole-5-acetic acid also inhibits carrageenin-induced edema in the rat.[3] Of the 75 related compounds that were synthesized and studied using this assay procedure, only two compounds were found to possess a potency comparable with indomethacin.

Chemistry. The bulk of the compounds were synthesized by the Hantzsch method, which involved reacting together the appropriate thioamide and α-bromo ketone in a solvent. In the course of this work three different solvent systems were investigated.

Initially, we were also interested in testing esters of the acids. Therefore, in some cases, for example, **1**, **10**, and **27**, the reactants were heated together in refluxing EtOH to give the esters **46**, **49**, and **48**, which were then hydrolyzed to the corresponding acids. Although this route gave reasonable yields, some esters were difficult to separate from starting materials and unwanted side products.

It was decided to obtain the acids directly by heating the reactants in *i*-PrOH at 60° in the presence of Na_2CO_3 essentially according to Knott.[4] Compounds **2–7**, **9**, and **13–22**, for example, were prepared in this way but yields were usually below 50%. It was then found that better yields and cleaner reactions were obtained when the reactants were heated in DMF at 70° without the presence of sodium carbonate, as illustrated for compounds **24–26** and **29–41**.

Derivatives of the acids, such as the amides **52** and **53** and the hydroxamic acid **54**, were prepared in the usual way as indicated in the footnotes of Table II. The α-propionic acid **61** was prepared from the appropriate bromoketo acid but the α-propionic acid **62** was prepared *via* alkylation of the ester **47** with CH_3I, essentially according to Kenyon, Kaiser, and Hauser,[5] and subsequent hydrolysis. The tetrazole **63** was obtained by treating the corresponding acetonitrile derivative **90**, prepared by dehydration of the amide **53**, with NaN_3 in DMF.

Structure-Activity Relationships. Analogs of **1** obtained by substitution in one or both of the phenyl rings are detailed in Table I together with the results on the carrageenin-induced edema test. Because of the variability inherent in this assay procedure, the ED_{40} of each compound is expressed as being within one of the six ranges detailed in the footnotes of Table I. The compounds were prepared essentially in the order shown. The objective was to establish the structure–activity pattern for each phenyl ring and from these data to prepare the appropriate polysubstituted compounds.

Substitution in the 2-phenyl ring resulted in a reduction of activity (compared to **1**) when the group was in the 2 position (**2**, **6**, **9**). When the group was in the 3 or 4 position activity was usually retained. In two cases, however, namely the 4-Cl (**7**) and the 4-$N(CH_3)_2$ (**11**), activity was slightly increased. Substitution in the 4-phenyl ring quickly revealed that the optimum group was chloro in