The ultraviolet absorption of the more active fractions showed a high intensity maximum at 290 mμ and a minimum at 260 mμ. The infrared absorption showed a broad hydroxyl band (3450 cm.$^{-1}$), a lactone (1740 cm.$^{-1}$), and an overall resemblance to a spectrum of podophyllotoxin (I).

Isolation of Podophyllotoxin(I).—The active lyophylized fractions were dissolved in water at pH 5, treated with half their weight of emulsin and allowed to stand 24 hours at 37°. The solutions were then extracted with chloroform in a continuous extractor. The chloroform was concentrated to dryness and the residue recrystallized from benzene, m.p. 110–113°. This melting point is in fair agreement with the value reported for podophyllotoxin (I) benzene hydrate (6, 7). The material was dried for 24 hours *in vacuo* at 100°, m.p. 180–182°. A mixed melting point with authentic podophyllotoxin (I) gave no depression and the infrared and ultraviolet spectra were identical.

Anal.—Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35; OCH_3, 22.47. Found: C, 63.88; H, 5.62; OCH_3, 22.18.

The yield of podophyllotoxin (I) from the dried needles based on spectrophotometric analysis was about 1.4 per cent.

The water solution from the chloroform extraction was treated with phenylhydrazone hydrochloride and sodium acetate. The resulting crystals of the phenylhydrazone gave no depression with the corresponding glucose derivative.

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Synthesis and Antifungal Activity of Anilides of Salicylic Acid and *o*-Coumaric Acid

By H. WAYNE SCHULTZ

A series of *o*-hydroxycinnamanilides and the corresponding series of salicylanilide derivatives were prepared and tested for antifungal activity. These compounds were investigated for the purpose of determining the effect of vinylogy and also the effect of substitution on the anilide rings. The results showed that all of the *o*-hydroxycinnamanilide derivatives had less antifungal activity than did their corresponding salicylanilide derivatives. Most of the compounds had some antifungal activity at the tested concentration of 0.5%; however, only two compounds had activity greater than salicylanilide. These compounds were the 3′-chloro- and the 4′-chloro-salicylanilides.

ALTHOUGH a large number of salicylanilide derivatives have been investigated for antifungal activity (1–10), the structural modifications have been generally limited to substitution on one or both of the aromatic rings. It was of interest to note that none of the reported investigations have been concerned with the vinylog derivatives, which are represented by the anilides of *o*-coumaric acid (*o*-hydroxycinnamanilides). Because of the principle of vinylogy (11), it appeared that such derivatives might possess activity similar to that of the related salicylanilide derivatives.

In this investigation a series of *o*-hydroxycinnamanilide derivatives and the corresponding series of salicylanilide derivatives were prepared and tested for antifungal activity. The compounds consisted of the free acids, the unsubstituted anilides, the 2′-, 3′-, and 4′-chloro-anilides, the 2′-, 3′-, and 4′-nitro-anilides and the 2′-, 3′-, and 4′-methyl-anilides. These derivatives not only provided the possibility of determining the effect of vinylogy but also the effect of substitution in varying positions on the anilide rings.

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EXPERIMENTAL

Synthesis of *o*-Hydroxycinnamanilides

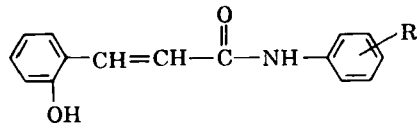
***o*-Coumaric Acid.**—From 32.1 Gm. (0.22 mole) of coumarin treated with 400 ml. of 8% sodium hydroxide and 4 Gm. of yellow mercuric oxide according to the procedure of Seshadri and Rao (12), there was obtained 27.5 Gm. (76%) of coumaric acid having a m.p. of 208°. This material was used in the following reaction.

***o*-Acetoxycinnamic Acid (13).**—A mixture of 27.5 Gm. (0.17 mole) of *o*-coumaric acid, 7.0 Gm. (0.85 mole) of sodium acetate and 90 ml. (0.95 mole) of acetic anhydride was heated on a steam bath for 7 hours. After cooling to room temperature, the reaction mixture was poured into 1 liter of ice and water and allowed to stand overnight. The white crystalline material was separated and washed with cold water and dried in air to give 33.0 Gm. of product (94% yield). A sample after recrystallization from benzene gave m.p. 153–154°.

***o*-Acetoxycinnamoyl Chloride (14).**—A mixture of 10.3 Gm. (0.05 mole) of *o*-acetoxycinnamic acid, 11.8 Gm. (0.1 mole) of thionyl chloride and 15 ml. benzene was heated under reflux for $^1/_2$ hour and cooled to room temperature. The solvent and excess thionyl chloride was removed *in vacuo* with gentle heating. Upon standing, a white crystalline material resulted.

4′-Methyl-2-Hydroxycinnamanilide.—In a typical example, the above *o*-acetoxycinnamoyl chloride

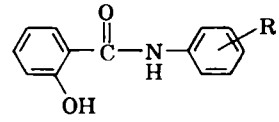
TABLE I.—*o*-HYDROXYCINNAMANILIDES



R	Formula	M.p.,[a] °C.	Yield,[b] %	Carbon,[c] % Calcd.	Carbon,[c] % Found	Hydrogen,[c] % Calcd.	Hydrogen,[c] % Found	Recryst. Solvent
H	$C_{15}H_{13}NO_2$	186–187	69	75.31	74.87	5.44	5.40	EtOH-H_2O
2′-Cl	$C_{15}H_{12}NClO_2$	174–175	49	65.81	66.02	4.39	4.16	EtOH-H_2O
3′-Cl	$C_{15}H_{12}NClO_2$	187–188	55	65.81	65.86	4.39	4.47	EtOH-H_2O
4′-Cl	$C_{15}H_{12}NClO_2$	235–236	63	65.81	65.52	4.39	4.31	EtOH-H_2O
2′-NO_2	$C_{15}H_{12}N_2O_4$	211–212	40	63.39	63.02	4.23	4.12	EtOH-H_2O
3′-NO_2	$C_{15}H_{12}N_2O_4$	237–238	43	63.39	63.78	4.23	4.31	EtOH-H_2O
4′-NO_2	$C_{15}H_{12}N_2O_4$	245–246	61	63.39	63.20	4.23	4.09	Pyridine-H_2O
2′-CH_3	$C_{16}H_{15}NO_2$	171–172	78	75.90	75.42	5.93	6.01	EtOH-H_2O
3′-CH_3	$C_{16}H_{15}NO_2$	170–171	57	75.90	75.51	5.93	5.92	EtOH-H_2O
4′-CH_3	$C_{16}H_{15}NO_2$	223–224	60	75.90	75.59	5.93	6.10	EtOH-H_2O

[a] Melting points are uncorrected and were obtained in capillary tubes. [b] Yield based on *o*-hydroxycinnamic acid. [c] Analyses by Weiler and Strauss, Oxford, Eng.

TABLE II.—SALICYLANILIDES



R	Formula	M.p.,[a] °C.	Yield,[b] %	Carbon,[c] % Calcd.	Carbon,[c] % Found	Hydrogen,[c] % Calcd.	Hydrogen,[c] % Found	Recryst. Solvent
H	$C_{13}H_{11}NO_2$	136–137[d]	79	73.24	73.19	5.16	5.10	EtOH-H_2O
2′-Cl	$C_{13}H_{10}ClNO_2$	165–166[e]	71	63.03	63.20	4.04	4.25	EtOH-H_2O
3′-Cl	$C_{13}H_{10}ClNO_2$	171–172[f]	78	63.03	62.90	4.04	4.11	EtOH-H_2O
4′-Cl	$C_{13}H_{10}ClNO_2$	167–168[g]	83	63.03	63.27	4.04	4.13	EtOH-H_2O
2′-NO_2	$C_{13}H_{10}N_2O_4$	155–157[h]	70	60.47	60.34	3.87	3.75	EtOH-H_2O
3′-NO_2	$C_{13}H_{10}N_2O_4$	216–217[i]	75	60.47	60.29	3.87	3.79	EtOH-H_2O
4′-NO_2	$C_{13}H_{10}N_2O_4$	228–229[j]	77	60.47	60.23	3.87	3.70	MeOH-H_2O
2′-CH_3	$C_{14}H_{13}NO_2$	142–143[k]	62	74.01	74.07	5.73	5.77	Benzene-Ligroin
3′-CH_3	$C_{14}H_{13}NO_2$	135–136[l]	79	74.01	73.93	5.73	5.71	EtOH-H_2O
4′-CH_3	$C_{14}H_{13}NO_2$	155–156[m]	75	74.01	74.11	5.73	5.81	EtOH-H_2O

[a] Melting points are uncorrected and were obtained in capillary tubes. [b] Yield based on salicylic acid. [c] Analyses by Weiler and Strauss, Oxford, Eng. [d] Reported m.p. 136–138° (16). [e] Reported m.p. 168° (17); 167° (10). [f] Reported m.p. 172° (17). [g] Reported m.p. 168–169° (18); 167–168° (4). [h] Reported m.p. 155° (19); 154° (20). [i] Reported m.p. 219° (19); 217–218° (20). [j] Reported m.p. 231° (21); 229–230° (20). [k] Reported m.p. 145° (19); 143–144° (22). [l] Reported m.p. 135–136° (19). [m] Reported m.p. 155–156° (20); 153–156° (23).

(11.2 Gm., 0.05 mole) was dissolved in 25 ml. benzene and a solution of 10.7 Gm. (0.1 mole) of *p*-toluidine in 25 ml. benzene was added slowly with stirring. The resultant mixture was refluxed for 15 minutes on a steam bath and then placed in an evaporating dish and allowed to evaporate to dryness. The solid residue was finely powdered, mixed with 300 ml. of 3% hydrochloric acid, and allowed to stand overnight. The solid material was separated with filtration and washed well with water. This material was dissolved in 150 ml. of 0.1 *N* sodium hydroxide solution and refluxed for 2 hours. After cooling to room temperature, the solution was acidified with hydrochloric acid and diluted with 200 ml. of water. The solid was collected, washed with water, and recrystallized from aqueous ethanol to give 8.1 Gm. of light tan crystals, m.p. 223–234°.

Synthesis of Salicylanilides

2′-Chlorosalicylanilide.—In a typical example a mixture of 10.4 Gm. (0.075 mole) of salicylic acid, 17.7 Gm. (0.15 mole) thionyl chloride, and 15 ml. benzene was refluxed for ½ hour and then cooled to room temperature. The solvent and excess thionyl chloride were removed *in vacuo* with gentle heating. The product was dissolved in 25 ml. benzene and a solution of 19.1 Gm. (0.15 mole) of *o*-chloroaniline in 25 ml. benzene was added slowly with stirring. The resultant mixture was refluxed for 15 minutes on a steam bath and then placed in an evaporating dish and allowed to evaporate to dryness. The solid residue was finely powdered, mixed with 300 ml. of 3% hydrochloric acid, and allowed to stand overnight. The solid material was separated with filtration and washed well with water. Upon recrystallization from aqueous ethanol there was obtained 13.2 Gm. (71%) of white crystals, m.p. 165–166°.

Antifungal Activity

The compounds were tested for their ability to inhibit the growth of three pathogenic species of fungi using a procedure similar to that described by Kligman and Rosenweig (15). Petri dishes containing sterile Sabouraud's agar were innoculated with the test organisms and a sterile filter paper disk was placed in the center of each dish. Test

TABLE III.—MINIMUM WIDTH[a] OF ZONES OF INHIBITION, IN MILLIMETERS, OF 0.5% SOLUTIONS

	T. mentagrophytes	*T. rubrum*	*E. floccosum*
2-Hydroxycinnamanilide	14	25	19
Salicylanilide	39	61	53
2′-Chloro-2-hydroxycinnamanilide	17	41	23
2′-Chlorosalicylanilide	20	51	33
3′-Chloro-2-hydroxycinnamanilide	21	36	25
3′-Chlorosalicylanilide	22	71	48
4′-Chloro-2-hydroxycinnamanilide	0	0	0
4′-Chlorosalicylanilide	35	76	61
2′-Nitro-2-hydroxycinnamanilide	0	0	18
2′-Nitrosalicylanilide	13	24	16
3′-Nitro-2-hydroxycinnamanilide	0	0	0
3′-Nitrosalicylanilide	13	34	26
4′-Nitro-2-hydroxycinnamanilide	0	0	16
4′-Nitrosalicylanilide	21	60	38
2′-Methyl-2-hydroxycinnamanilide	16	24	22
2′-Methylsalicylanilide	23	54	32
3′-Methyl-2-hydroxycinnamanilide	26	33	30
3′-Methylsalicylanilide	27	57	37
4′-Methyl-2-hydroxycinnamanilide	14	0	0
4′-Methylsalicylanilide	17	46	35
2-Hydroxycinnamic acid	0	0	0
Salicylic acid	0	0	14

[a] Average of two measurements.

solutions of the compounds at 0.5% concentration were prepared using alcohol or acetone as the solvent. Alcohol (95% w/v) was used for all of the compounds except the 2′-, 3′-, and 4′-nitro-2-hydroxycinnamanilide derivatives and the 3′- and 4′-nitrosalicylanilide derivatives. For these compounds, which were insoluble in alcohol at the desired concentration, acetone was used as the solvent. To each filter paper disk, 0.8 ml. of a test solution was applied by means of a pipet. Two plates of an organism were treated with each test solution. The pure solvents, alcohol and acetone, were also tested. The plates were incubated at 28° for 72 hours. At the end of this time the diameter of the zone of inhibition was measured.

DISCUSSION

As shown in Table III, all of the *o*-hydroxycinnamanilide derivatives had less antifungal activity than did their corresponding salicylanilide derivatives. Most of the compounds had some antifungal activity at the tested concentration of 0.5%; however, only two compounds had activity greater than salicylanilide. These compounds were the 3′-chloro- and the 4′-chloro-salicylanilides. Of these two, the 4′-chlorosalicylanilide derivative had greater activity against *T. rubrum* and *E. floccosum* than salicylanilide and had an activity against *T. mentagrophytes* which was almost equal to that of salicylanilide.

Among the substituted chloro- and nitro- salicylanilide derivatives it was found that the 4′- position gave the greatest antifungal activity, while the 3′-position was the most effective of the methyl derivatives. The anilide derivatives of *o*-hydroxycinnamic acid did not show this overall consistency in relationship between position and activity. The results further showed that the activity of salicylanilide was enhanced only by the substitution of a 4′-chloro group on the anilide ring, while with its vinylog, *o*-hydroxycinnamanilide, substitution of either 2′-chloro, 3′-chloro, 2′-methyl, or 3′-methyl enhanced its activity.

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