Synthesis of Halogen Substituted Derivatives of 4,4-Bis(4-hydroxyphenyl)pentanoic Acid and Their Antifungal Properties

By RASHMIKANT M. PATEL*, DAVID P. CAREW, and JOHN L. LACH

Several derivatives of 4,4-bis(4-hydroxyphenyl)-pentanoic acid and 10-undecenoic hydroxamic acid were prepared. Their antifungal activity was tested against *Trichophyton mentagrophytes* (ATCC 9533). Six of the ten compounds tested exhibited greater activity than undecylenic acid under the conditions of the test.

MANY COMPOUNDS have been synthesized and evaluated for their antimicrobial activity. These include quinones, quaternary ammonium salts, sulfur containing derivatives, hydroxamic acids, halogenated phenols, and acid esters (1–6). Among these diverse chemical types, compounds having a phenol nucleus such as hexachlorophene, bithionol, and resorcinol have a prominent place in many soaps, ointment, and scrub lotions.

A previous study with the fungus *Trichophyton rubrum* and with streptococci indicated that diphenolic acid[1] [4,4-bis(4-hydroxyphenyl)pentanoic acid] possesses antifungal and antibacterial activity *in vitro* (7). Since diphenolic acid is a suitable intermediate for the preparation of other compounds with possible antifungal activity, it was of interest to prepare its halogenated derivatives and some of their esters.

Since some hydroxamic acid derivatives have been shown to exhibit antifungal activity, the hydroxamic acid of the widely used undecylenic acid was also prepared and evaluated for its antifungal property.

EXPERIMENTAL

4,4-Bis(3,5-dichloro-4-hydroxyphenyl)pentanoic Acid—A 1-L. round-bottom flask, fitted with an efficient condenser carrying a calcium chloride drying tube, was set up on a steam bath in a hood. In the flask were placed 28.6 Gm. (0.1 mole) of 4,4-bis-(4-hydroxyphenyl)pentanoic acid and 67.5 Gm. (0.5 mole) of sulfuryl chloride, and the mixture was warmed on the steam bath for 1 hr. Ten more milliliters of sulfuryl chloride was added to the reaction flask and the warming was continued for about 30 min. Excess sulfuryl chloride was removed by attaching a water pump through an empty safety flask to the reaction flask. The crude product was washed with boiling water, dried at 60°, and recrystallized from benzene.

4,4-Bis(3,5-dibromo-4-hydroxyphenyl)pentanoic Acid—In a 1-L. three-necked flask, fitted with a mechanical stirrer, dropping funnel, and a condenser, were placed 28.6 Gm. (0.10 mole) of diphenolic acid and 360.0 ml. of glacial acetic acid. A solution of 40.0 ml. (1.6 mole) of bromine in 40.0 ml. of glacial acetic acid was placed in the dropping funnel and added to the diphenolic acid solution during a 30-min. period and stirred continuously for 22 hr. The flask was heated on a steam bath and air was passed through the side neck to remove excess bromine. The mixture was poured into a beaker containing a saturated solution of sodium bisulfite in water. The resulting semisolid mass was washed with cold water and placed in a beaker containing boiling water, and stirred until the semisolid mass solidified. The hot water was decanted, and the crude product was dried at 60°, and recrystallized from benzene.

Esterification of Halogenated Acids—Methyl and propyl esters were prepared according to the following procedure. A mixture of purified halogenated diphenolic acid (0.05 mole), appropriate alcohol (75 ml.), and concentrated sulfuric acid (5 ml.) was refluxed for 5 hr. The reaction mixture was poured into a beaker containing ice, neutralized with a saturated solution of sodium bicarbonate, filtered, and washed with water. The crude ester was vacuum dried and recrystallized from benzene.

N-Decyl and cyclohexyl esters were prepared by reaction of appropriate acid chloride with the alcohol under consideration. The general procedure was as follows. In a 250-ml. conical flask, fitted with a condenser, was placed 0.05 mole of halogenated acid and 59.0 Gm. (0.5 mole) of thionyl chloride. The reaction mixture was heated on a steam bath for 0.5 hr. At the end of the reaction period, 50.0 ml. of dry benzene was placed in the flask and excess thionyl chloride distilled under vacuum. A solution of 0.05 mole of the appropriate alcohol in 30.0 ml. benzene was added to the flask containing acid chloride and refluxed for 1 hr. After removing benzene, the crude product was treated with a saturated solution of sodium bicarbonate, filtered, washed, dried, and recrystallized from an acetic acid–water mixture. The recrystallization solvents, melting points, yields, and elemental analyses are given in Table I.

10-Undecenoic Hydroxamic Acid—Separate solutions of 14.0 Gm. (0.2 mole) of hydroxylamine hydrochloride in 80.0 ml. of methyl alcohol and 16.8 Gm. (0.3 mole) of potassium hydroxide in 40.0 ml. of methyl alcohol were prepared at the boiling point of the solvent. Each was cooled to 35° and the one containing alkali was added with shaking to hydroxylamine hydrochloride solution. Any excessive rise of temperature was prevented by occasional cooling in an ice bath. After the alkali had been added the mixture was allowed to stand

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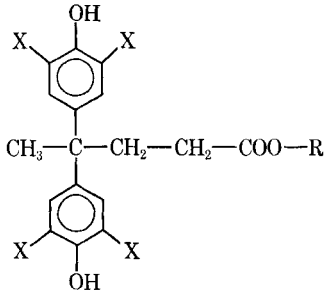
[1] Obtained from S. C. Johnson & Son, Inc., Racine, Wis.

TABLE I—DERIVATIVES OF 4,4-BIS(4-HYDROXYPHENYL)PENTANOIC ACID



No.	X	R	% Yield	M.p., °C.	Recrystn. Solvent	Anal., % Calcd. C	Calcd. H	Calcd. X	Found[a] C	Found[a] H	Found[a] X
1[b]	Cl	H	38	182–183	Benzene	48.13	3.30	33.45	48.33	3.45	32.24
2[b]	Br	H	62	159–160	Benzene	33.90	2.33	53.13	34.15	2.25	51.90
3	Cl	Me	71	139–140	Benzene	49.36	3.68	...	49.50	3.84	...
4	Br	Me	54	122–123	Benzene	35.10	2.62	...	35.36	2.86	...
5	Cl	*n*-Pr	32	112–113	Benzene	51.52	4.32	...	51.78	4.11	...
6	Br	*n*-Pr	25	123–124	Benzene–benzine	37.30	3.13	...	37.95	3.01	...
7	Br	Cyclohexyl	29	93–94	Acetic acid–water	40.37	3.53	...	40.30	3.65	...
8	Br	Decyl	20	84–85	Acetic acid–water	43.69	4.60	...	43.69	4.58	...

[a] C, H analysis was done at Clark's Microanalytical Laboratory; halogen by Parr bomb method. [b] Now sold by Emery Laboratories, Cincinnati, Ohio.

in an ice bath for 5 min. to insure complete precipitation of potassium chloride. Then 19.8 Gm. (0.1 mole) of methyl 10-undecenoate was added with thorough shaking and the mixture was filtered immediately with suction. The filtrate was placed in a conical flask and allowed to stand at room temperature. After 2 days, crystals were filtered, washed with a minimum amount of absolute ethyl alcohol, and air dried. A mixture of the potassium salt and 100.0 ml. of dilute acetic acid was stirred and heated on a steam bath until a clear solution was obtained. It was allowed to cool at room temperature and finally placed in an ice bath. The precipitated hydroxamic acid was recrystallized twice from glacial acetic acid–water, whereupon colorless crystals were obtained. The yield was 11.0 Gm. (55%), m.p. 72–73°.

Anal.—Calcd. for $C_{11}H_{21}NO_2$: C, 66.32; H, 10.62; N, 7.02. Found: C, 67.03; H, 10.92; N, 7.22.

Determination of Fungicidal Activity—A study of the fungicidal properties of the compounds whose synthesis has just been reported was conducted. The testing procedure was a modification of the methods of Burlingame (8), Golden (9), and Reddish (10). The test organism *T. mentagrophytes* (ATCC 9533) was used exclusively. Since the compounds to be tested for fungicidal activity were insoluble in water, ethanolic solutions were prepared. Solutions of 0.1%, 0.5%, and 1.0% in ethanol (95%) were made. Ten tests for each of the three concentrations were run for each compound.

Test Procedures—Petri dishes containing 20 ml. Sabouraud dextrose agar were inoculated with a culture of *T. mentagrophytes*. The inoculated plates were incubated at a temperature of 28° for 15 days. On the day of the test 1-cm. disks of agar and fungus were removed from the Petri dishes with a sterile cork borer (10 mm. diameter). Each disk was immediately aseptically transferred to a test tube containing 10 ml. of a particular concentration of the solution of the compound under test. The test tube was agitated slowly for 2 min. and the disk was transferred to a test tube containing 10 ml. sterile Sabouraud dextrose broth. This tube was agitated slightly for a period of 3 min. to free the mycelia of any soluble or miscible material. The disk was then transferred to a test tube containing 10 ml. of a 30% aqueous acetone solution where it was kept for 5 min. to remove any traces of adhering antifungal compound. The disk was transferred to a test tube of sterile Sabouraud dextrose broth for a 2-min. period in order to remove any traces of acetone. Finally the disk was removed from the broth and placed culture side down on the surface of a sterile slant of Sabouraud dextrose agar contained in a 25 × 150 mm. test tube. The inoculated slants were incubated at a temperature of 28° for 25 days. Controls were run along with each compound and these consisted of agar-fungus disks which were treated as above except that the initial alcoholic solution did not contain any chemical compound being tested. Evidence of growth in control tubes was always evident in a few days after inoculation. In addition to the newly synthesized compounds, the known antifungal agent undecylenic acid was used for comparison. Since the testing procedure was designed to determine fungicidal activity, any evidence of fungal growth after a 25-day period was regarded as a negative result. Table II lists the results of the fungicidal testing procedure.

TABLE II—RESULTS OF FUNGICIDAL TESTING

Compd. No. or Name	No. of Tubes Exhibiting Growth After 25 Days[a] 0.1%	0.5%	1.0%
1	10	8	6
2	10	10	10
3	4	4	3
4	6	4	4
5	4	4	0
6	6	4	2
7	8	6	6
8	4	4	4
Diphenolic acid	10	3	3
Undecylenic acid	8	4	2
10-Undecenoic hydroxamic acid	2	0	0

[a] The maximum number of tubes exhibiting growth for each concentration is 10.

CONCLUSION

From Table II it can be seen that 10-undecenoic hydroxamic acid exhibited the greatest amount of fungicidal activity. Propyl 4,4-bis(3,5-dichloro-4-hydroxyphenyl) pentanoate was the next most active fungicidal compound tested. Six of the ten compounds tested exhibited greater activity than undecylenic acid under the conditions of the test.

The undecylenic hydroxamic acid, because of its activity against *T. mentagrophytes*, was further tested and showed good activity against the following organisms: *Microsporum audouini* ATCC 9079, *Microsporum canis* ATCC 10241, *Trichophyton mentagrophytes (gypseum)* ATCC 9129, *Trichophyton mentagrophytes (interdigitale)* ATCC 9972, *Candida albicans* ATCC 10231, *Trichophyton rubrum* ATCC 10281, *Stemphylium solani* ATCC 11128, and *Stemphylium species* ATCC 9569.

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Activity of Different Segments of Rabbit Gastrointestinal Tract

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Five different segments of the rabbit intestinal tract, (duodenum, jejunum, ileum, ascending and descending colon), were used to record the normal activity of these segments using an isolated muscle bath technique. Kymograph recordings and transducer-recorder recordings were compared. Contractions per minute, interval between contractions, and amplitude of contractions were observed and evaluated. In the transducer recorded series, *t* tests indicated statistically significant difference for all comparisons of contractions per minute. From 60 comparisons of normal rabbit intestinal activity using *t* tests, only 5 comparisons were not found to be significantly different at *P* 0.05.

THE WEIGHTS and linear measurements of several segments of the gastrointestinal tract of the rabbit have been investigated by Latimer and Sawin (1). Other tissues and organs have been studied (2–5), but the variations in the normal activity of different segments of the rabbit gut so often employed in drug testing and drug experimentation require delineation.

It was, therefore, the purpose of this study to record some of the variations observed in the different segments of the rabbit intestine and subject them to statistical evaluation for reference when a similar preparation was used experimentally. Two systems, typical kymograph and transducer systems, were employed to further delineate differences in observations of activity of rabbit intestinal segments.

EXPERIMENTAL

Adult, albino rabbits were anesthetized with ether and 2–3 cm. segments of duodenum, jejunum, ileum, ascending and descending colon were expediently removed. The segments were individually maintained in oxygenated Tyrode's solution in a constant-temperature bath at 38° prior to their placement in the isolated muscle bath. The Tyrode's solution contained sodium chloride 0.8%, potassium chloride 0.02%, calcium chloride 0.02%, magnesium chloride 0.01%, sodium bicarbonate 0.1%, sodium diphosphate 0.005%, and glucose 0.1%.

The first series of experiments utilized constant-temperature muscle baths and kymographs. Tyrode's solution (100 ml.) was used to bathe the segment; temperature was maintained at 38° and the preparation was oxygenated using an air flow bubbled through the solution. The segment was attached to the muscle hooks, one stationary and the other being connected to a lever and stylus, such that the segment was completely submerged in the Tyrode's solution. The muscle lever was made to record on a slowly rotating carbon black kymograph drum. A synchronous timer recorded the time on the same tracing. The only constant tension placed upon the segment was that of the weight of the lever which in turn was centered on its fulcrum and

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