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CONSTITUENTS OF THE MARINE ANNELID *THELEPUS SETOSUS*

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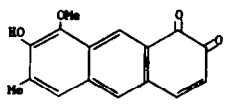
Abstract—From the marine annelid *Thelepus setosus* we have isolated isovaleramide, 3,5-dibromo-4-hydroxybenzyl alchol (2), the corresponding aldehyde (3), bis(3,5-dibromo-4-hydroxyphenyl)methane (4), 2,4'-dihydroxy-5-hydroxymethyl-3,3',5'-tribromodiphenylmethane (thelephenol, 6), and dienone 5 (thelepin), which shares with the antimicrobial agent griseofulvin (13) structural similarity and antifungal activity.

The intriguing *ortho*-anthraquinone structure of hallachrome, which is the epidermal pigment of a marine annelid (1),[1] coupled with our interest in marine pig-



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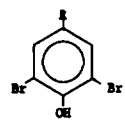
ments,[2-4] prompted us to examine several annelid species, including *Marphysa sanguinea*, which is a member of the family Eunicidae to which the hallachrome (1) elaborator, *Halla parthenopeia*, belongs. Electronic spectral examination of chloroform extracts of our annelids after fractionation by TLC revealed at once that no *o*-anthraquinones were present. Instead, the spectra which we scanned were typically those of carotenes or porphyrins, or else appeared to be quite unlike those of conventional chromophores encountered in natural pigments. The tube-dwelling polychaete *Thelepus setosus* (Quatrefages, 1865), Family Terebellidae, seemed to elaborate all three of the above pigment types, which we have not yet studied in detail. The chloroform extract of this annelid, on silicic acid chromatography, yielded several fractions that contained bromo constituents. We reported the purification and structural elucidation of five bromophenol related compounds (2–6) in a preliminary communication.[5] Subsequent leaching of the animals with acetone resulted in the isolation of one further metabolite, isovaleramide. Its parent acid, which is a widely distributed constituent of terrestrial plants, has recently been identified as a mammalian pheromone in the scent glands of the male pronghorn deer (*Antilocapra americana*).[6]

For the isolation of the bromophenol related metabolites we treated the freeze-dried animals with chloroform in a blender and chromatographed the residual oil on Bio-Sil. From light petroleum-chloroform eluates, after further TLC separation, we isolated 3,5,-dibromo-4-hydroxybenzaldehyde (3) and bis(3,5-dibromo-4-hydroxyphenyl)methane (4). We deduced their structures from spectral properties and confirmed them by synthesis.

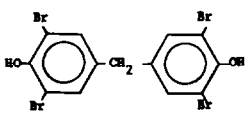
From the pure chloroform eluates, followed by TLC, we isolated 3,5-dibromo-4-hydroxybenzyl alcohol (2), readily identified by comparison with a synthetic sample; compound 5, which we have named thelepin; and finally a small amount of 2,4' - dihydroxy - 5 - hydroxymethyl - 3,3',5' - tribromodiphenylmethane (6), coveniently designated as thelephenol.

Thelepin (5) is a pale yellow crystalline compound, m.p. 202–203° (dec). Its mass spectrum showed the presence of three bromine atoms for a composition of $C_{14}H_9Br_3O_3$. One O atom was assigned to a conjugated CO (ν_{max} 1690 cm^{-1}), another to a benzyl alcohol on the basis of one exchangeable proton at δ 2·87 and a broad two proton singlet at δ 4·56, which was shifted to δ 4·94 in thelepin monoacetate (7), m.p. 190°, in full analogy with corresponding PMR signals in 2. The third oxygen atom was inferred to be the ether because of lack of direct spectral evidence.

Two finely split multiplets at δ 7·26 and 7·36 in the PMR spectrum of 5 pointed to a tetrasubstituted benzene. This system and the conjugated carbonyl accounted for six of the nine unsaturations in 5. Catalytic hydrogenation over Pd–C appeared to involve uptake of 5 moles of hydrogen and yielded a single product, m.p. 135–136°, which lacked bromine and carbonyl, but by its composition of $C_{14}H_{14}O_2$ appeared to have retained all but one element of unsaturation. This initially puzzling transformation provided a clue to the thelepin structure by its apparent analogy with the hydrogenation of bromodienone 8 to phenol 9.[7] Spectral data of our hydrogenation product suggested a structure of 2,4' - dihydroxy - 5 - methyl-



2, R = CH_2OH
3, R = CHO



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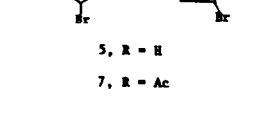
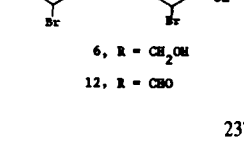
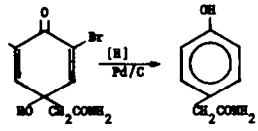


5, R = H
7, R = Ac



6, R = CH_2OH
12, R = CHO



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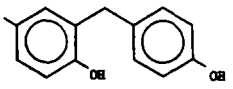
diphenyl methane (**10**), which we confirmed by comparison with synthetic **10** and with the corresponding dimethyl ethers.



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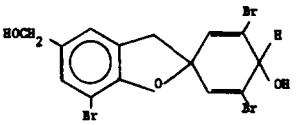

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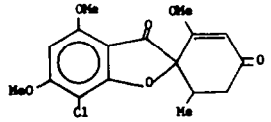
All of these data, and the fact that the annelid contained compounds **2** and **3**, constituted good evidence for structure **5** of thelepin. We corroborated this structure by sodium borohydride reduction, which led to equal amounts of **6** and **11**. We synthesized **6** (2,4' - dihydroxy - 5 - hydroxymethyl - 3,3',5' - tribromodiphenylmethane or thelephenol) by condensing 3 - bromo - 4 - hydroxybenzaldehyde and 3,5 - dibromo- 4 - hydroxybenzyl alcohol (**2**) *in* polyphosphoric acid. This reaction led to **12**, which was reduced to **6** by sodium borohydride. We made two unsuccessful attempts to transform **6** into thelepin (**5**) itself by oxidative coupling with ferricyanide[8] or lead dioxide.[9] The spectral properties of the second borohydride product were in accord with dienol structure **11**, which we confirmed by acid-catalyzed rearrangement of **11** to **6**, which we had already synthesized (*vide supra*).

Compound **6**, m.p. 180–182°, is also a natural metabolite of the annelid. We isolated it it small quantity (0·001%) from the tail fractions of the chloroform eluate. It seems reasonable to assume that **6** or a debromo analog is the biological precursor of thelepin in the annelid. The co-occurrence of **5** and **6** with **2**, **3**, and **4** makes this an attractive hypothesis, The monocyclic bromophenols **2** and **3*** are known constituents of a number of red algae,[10] but **4**, **5**, **6** were not previously reported.

Thelepin (**5**) exhibits antifungal activity at a level comparable with griseofulvin (**13**), which was first isolated from the microorganism *Penicillium griseofulvum*[11] and to which it bears considerable structural resemblance.



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EXPERIMENTAL

All m.ps were determined on a Fisher–Johns block and are uncorrected. Combustion analyses were performed by University of California Chemical Analytical Services, Berkeley, CA. UV spectra were measured on a Beckman DK-2 and IR spectra on a Beckman IR-10 instrument. Mass spectra were recorded by Sr. M. R. Brennan on A Hitachi–Perkin–Elmer RMU-6D and NMR spectra by Mr. J. Loo on a Varian HA-100 instrument. Silica gel HF 254 + 366 (E. Merck) was used for all TLC.

Extraction and isolation. T. setosus (1 kg) was collected at Kaneohe Bay, Oahu, in June 1973, detached from the tubes, and freeze-dried for 4 d. The dry material (375 g) was treated with chloroform (2·5 l) in a blender, filtered, and washed with fresh chloroform (500 ml). Evaporation of the solvent left 24 g dark-brown oil. The oil was chromatographed on a column of Bio-Sil A (420 g). The column was eluted yielding 61 fractions, 200 ml each: fractions 1–12 with petroleum ether, 13–20 with light petroleum-chloroform 5:1, proceeding to 10:3 for 21–25, 2:1 for 26–32, 1:1 for 33–40, and pure chloroform for 41–61. Fractions 24–27 and 47–58 contained the bromo compounds.

3,5-Dibromo-4-hydroxybenzaldehyde (**3**). The solid residue of fractions 24–27 was separated by TLC with acetone-chloroform (1:10) into two major components (R_F 0·18 and 0·72). The product from the band at R_F 0·18 was sublimed *in vacuo* and recrystallized from chloroform to furnish aldehyde **3** (4·5 mg, 0·000 45%): m.p. 182–186°, mixture m.p. with a synthetic sample 182–187°; M^+ at *m/e* 282 (43), 280 (89), and 278 (50) and M-1 at *m/e* 281 (58), 279 (100), and 277 (50%). IR spectrum was identical with that of a synthetic sample.

Synthesis. To a solution of *p*-hydroxybenzaldehyde (200 mg) in aq EtOH (1:1, 4 ml) was added bromine-water soln, prepared by dissolving Br_2 (1 g) and KBr (1·6 g) in water (10 ml), until the color of Br_2 no longer disappeared. Water (5 ml) was added, and ppts was filtered off and thoroughly washed with water. The product was recrystallized from MeOH to yield **3** (320 mg), white needles, m.p. 186–187° (lit[12] m.p. 181°).

Bis-(3,5-dibromo-4-hydroxyphenyl)methane (**4**). The second component (R_F 0·72) of fractions 24–27 was recrystallized from benzene to yield **4** (12·4 mg, 0·00124%) as colorless plates: m.p. 230–232°; ν_{max} (KBr) 3440, 1618, 1556, 1473, 1403, 1304, 1273, 1237, 1197, 1142, and 727 cm^{-1}; λ_{max} (MeOH) 285 (log ϵ 4·26), and 314 nm (3·95); δ (acetone-d_6) 3·89 (2H, s) and 7·45 (4H, s); *m/e* 520 (18), 518 (67), 516 (100), 514 (69), 512 (19%). (Found: C, 30·3; H, 1·55; Br, 61·95. $C_{13}H_8Br_4O_2$ requires: C, 30·4; H, 1·55; Br, 61·8%).

Synthesis. 4,4'-Dihydroxydiphenylmethane was prepared by heating phenol and formaldehyde in the presence of urea and hydrochloric acid, according to the method of Ishikawa *et al.*[13] After drying, the product (1.5 g) was twice recrystallized from aq EtOH and from benzene to yield long plates, m.p. 230–232° (lit.[14] m.p. 224–226°). IR, NMR, and mass spectra were identical to those of the natural product.

3,5-Dibromo-4-hydroxybenzyl alcohol (**2**). The residue of fractions 47–52 was chromatographed on silica gel plates with acetone-chloroform (1:10). Recrystallization of the major product at R_F 0·4 from chloroform afforded **2** (500 mg, 0·05%): m.p. 115–116°, mixture m.p. with a synthetic sample showed no depression; ν_{max} (KBr) 3480, 3300, 2880, 1475, 1410, 1322, 1278, 1243, and 1163 cm^{-1}; δ (acetone-d_6) 4·56 (2H, s) and 7·51 (2h, s); *m/e* 284 (32), 282 (68), 280 (36), 203 (60), 201 (60), 174 (34), 172 (34), and 94 (100%). IR spectrum was identical with that of a synthetic sample.

Synthesis. A soln of **3** (115 mg) in 95% EtOH (5 ml) was dropwise added to a soln of $NaBH_4$ (30 mg) in EtOH (3 ml). The mixture was stirred at room temp. for 30 min, decomposed with dil HCl-ice, and thrice extracted with ether. The ethereal soln was dried ($MgSO^4$) and evaporated to furnish a solid which on recrystallization from chloroform gave a product (85 mg), m.p. 110–115°. The product was purified by TLC on silica gel plates with acetone-chloroform (1:10) followed by recrystallization from chloroform to afford a pure sample of **2**, m.p. 116–117° (lit[15] m.p. 116–117°).

Thelepin (**5**). The residue of fractions 53–57 was separated by TLC on silica gel plates with acetone-chloroform (1:10). The product from the major band at R_F 0·36 was recrystallized from MeOH to furnish **5** (130 mg, 0·013%) as fine pale yellow crystals, m.p. 199–201° (dec). Another recrystallization from the same solvent gave a pure sample of **5**: m.p. 202–203° (dec); ν_{max} (KBr) 3430, 3045, 2910, 2865, 1685, 1605, 1468, 1440, 1312, 1263, 1207, 1109, 997, 963, 893, 853, 816, 756, and 690 cm^{-1}; λ_{max} (EtOH) 247 sh (log ϵ 4·09), 257 (4·12), 280 sh (3·72), and 288 sh nm (3·59); δ (acetone-d_6) 2·87 (br. s), 3·73 (2H, m), 4·56 (2H, s), 7·26 (1H, m), 7·36 (1H, m), and 7·75 (2H, s); *m/e* 468 (39), 466 (100), 464 (98), 462 (35), 452 (2), 450 (5), 448 (5), 446 (2), 387 (6), 385 (11), 383 (6), 369 (6), 367 (11), 365 (6), 341 (2), 339 (4), 337 (2), 328 (2), 326 (4), 324 (2), 306 (4), 305 (6), 304 (4), 303 (6), 278 (12), 277 (12), 276 (32), 275 (12), 274 (20), 261 (6), 259 (6), 243 (8), 241 (8), 197 (11), 196 (10), 169 (11), 168 (12), 152 (11), and 139 (29%). (Found: C, 36·3; H, 2·0; Br, 51·3. $C_{14}H_9Br_3O_3$ requires: C, 36·2; H, 1·95; Br, 51·55).

Thelepin monoacetate (**7**). A mixture of **5** (6·0 mg), Ac_2O (0·5 ml), and pyridine (0·5 ml) was stirred at room temp. for 5 hr. After excess reagents were removed *in vacuo*, the residue was

*In our preliminary account we erroneously stated that only **2** had been isolated previously.

chromatographed on a silica gel plate with chloroform to furnish a solid (3·3 mg), which on recrystallization from carbon tetrachloride afforded **7** as pale yellow needles: m.p. 190°; ν_{max} ($CHCl_3$) 1740, 1690, 1640, 1605, 1462, 1360, 1310, 1262, 1116, and 960 cm^{-1}; δ (CCl_4) 2·04 (3H, s), 3·52 (2H, m), 4·94 (2H, s), 7·13 (1H, m), 7·35 (1H, m), and 7·36 (2H, s); λ_{max} (EtOH) 244 (log ϵ 4·04), 256 (4·02), 280 sh (3·59), and 288 hs nm (3·48); *m/e* 510 (36), 508 (100), 506 (100), 504 (36%).

Catalytic hydrogenation of thelepin (**5**). Thelepin **5** (47 mg) in 95% EtOH (20 ml) was hydrogenated over 10% Pd/C (25 mg) with atmospheric H_2 at room temp. until the uptake (13·3 ml) ceased (1 hr). After filtration the soln was concentrated to yield solid residue which on recrystallization from chloroform-cyclohexane afforded 2,4' - *dihydroxy* - 5 - *methyldiphenylmethane* (**10**; 15 mg, 70%) as colorless plates: m.p. 135–136° (lit.[16] m.p. 135·5°); ν_{max} (KBr) 3320, 3020, 2920, 1612, 1600, 1502, 1427, 1107, 1094, 1010, 932, 902, 795, 764, and 730 cm^{-1}; λ_{max} (MeOH) 280 (log ϵ 3·70) and 283 sh nm (3·68); δ (acetone-d_6) 2·20 (3H, s), 3·15 (s, OH), 3·88 (2H, s), and 6·71–7·15 (7H, m); *m/e* 214 (77), 199 (10), 121 (100%).

Synthesis of 2,4'-*dihydroxy*-5-*methyldiphenylmethane* **10**). To a stirred mixture of *p*-cresol (11 g) and *p*-hydroxybenzyl alcohol (6·2 g) in MeOH (20 ml) was added dropwise conc H_2SO_4 (2 g) at 0°. The mixture was stirred at this temp. for 1 hr and an additional 1 hr at room temp., poured in ice-water, and extracted with ether. The ethereal soln was dried ($MgSO_4$), concentrated, and distilled *in vacuo* to remove unreacted reagents. The residue (glass) was sublimed at 130° (0·3 mm) to yield **10** (1·4 g, 13%) which on recrystallization from aq EtOH gave long plates, m.p. 137–138°. IR and NMR spectra were identical with those of **10** obtained by the reduction of **5**.

Reduction of thelepin with sodium borohydride. To a soln of **5** (8·5 mg) in 95% EtOH (2 ml) was added $NaBH_4$ (*ca.* 3 mg) in three portions. The mixture was stirred at room temp. for 25 min and acidified with 0·1 N HCl. After evaporating EtOH the ppts were filtered, washed with water, and dried to yield a mixture which showed three spots by TLC. Two major components (R_F 0·42) and 0·30) were isolated by TLC on a silica gel plate with MeOH-chloroform (3:100). The least polar band gave **6** (3·0 mg), the IR spectrum of which was identical with that of a synthetic sample of thelephenol. The second band furnished **11** (3·2 mg): m.p. 160–162°; λ_{max} (MeOH or MeOH-NaOH) 284 and 291 nm).

Synthesis of 2,4' - *dihydroxy* - 5 - *formyl* - 3,3',5' - *tribromodiphenylmethane* (**12**). A mixture of 3 - bromo- 4 - hydroxybenzaldehyde (201 mg), prepared from 4-hydroxybenzaldehyde according to Paal,[17] alcohol **2** (282 mg), and polyphosphoric acid (10 g) was blended in a beaker on a water-bath at 70–80° for 20 min. The mixture was dissolved by adding ice. The ppts, after filtration and thorough washing with water were chromatographed on silica gel plates with MeOH-chloroform (3:100). The least polar component was collected and recrystallized from MeOH to yield the aldehyde **12** (75 mg, 16%): m.p. 205–208°; ν_{max} (KBr) 3430, 1676, 1620 sh, 1594, 1565 sh, 1468, 1425, 1380, 1240, 1140, 1086, 860, and 724 cm^{-1}; δ (acetone-d_6) 3·37 (br, s), 4·15 (2H, s), 7·54 (2H, s), 7·83 (1H, d, J = 2·0 Hz), 8·05 (1H, d, J = 2·0 Hz), and 9·92 (1H, s); *m/e* 468 (10), 467 (35), 466 (25), 465 (98), 464 (25), 463 (100), 462 (11), 461 (35%).

Sodium borohydride reduction of **12** *to* **6**. Aldehyde **12** (23·5 mg) was dissolved by heating in EtOH (5 ml). After cooling, $NaBH_4$ (*ca* 10 mg) was added, a portion at a time. The soln was stirred for 5 min, acidified with 0·1 N HCl (5 ml), and water (2 ml) was added. The milky suspension was cooled on ice, and ppts were filtered, washed with water, and dried to yield **6** (21 mg) as white, fine crystals, m.p. 183–184°, λ_{max} (MeOH) 284 nm, λ_{max} (MeOH-NaOH) 306 nm. IR, NMR, and mass spectra were identical with those of the natural product. Analytical sample was recrystallized from aq EtOH to yield white needles, m.p. 183–184°. (Found C, 35·9; H, 2·25; Br, 51·25. $C_{14}H_{11}Br_3O_3$ requires: C, 36·0; H, 2·35; Br, 51·35).

Attempted Oxidation of thelephenol (**6**) *to thelepin* (**5**). A small amount of **6** was oxidized with potassium ferricyanide by the method of Day *et al.*[8] Analysis of the product mixture by TLC showed two spots, none of which was identical with that of thelepin. Thelephenol was next oxidized with lead dioxide by the procedure of Taub *et al.*[9] to furnish a mixture consisting of three components, none of which was identical with that of thelepin as shown by TLC.

Conversion of thelepinol (**11**) *to thelephenol* (**6**). Thelepinol **11** (*ca* 3 mg) in MeOH (2 ml) and 0·1 N HCl (2 drops) was heated for 3 hr. The product, which showed a spot identical to **6**, was purified by TLC on a silica gel plate with MeOH-chloroform (3:100) to yield a small amount of **6**. Its UV (MeOH and MeOH–NaOH) and IR spectra were identical with those of authentic **6**.

2,4' - *dihydroxy* - 5 - *hydroxymethyl* - 3,3',5' - *tribromodiphenylmethane* (**6**). Chromatography of the residue of fraction 58 on silica gel plates with MeOH-chloroform (3:100) gave a small amount of solid, R_F 0·24. The solid was rechromatographed in the same manner to furnish **6** (11 mg, 0·0011%) as white crystalline solid: m.p. 180–182°, mixture m.p. with authentic sample showed no depression; ν_{max} (KBr) 3440, 2935, 2875, 1612, 1555, 1472, 1425, 1404, 1307, 1235, 1142, 1095, 1000, 862, and 726 cm^{-1}; δ (acetone-d_6) 3·99 (2H, s), 4·55 (2h, s), 7·18 (1H, m), 7·41 (1H, m), and 7·43 (2H, s); *m/e* 4·70 (34), 468 (93), 466 (100), 464 (41), 452 (23), 450 (58), 448 (57), 446 (22), 439 (7), 437 (18), 435 (18), 433 (11), 389 (9), 387 (23), 385 (18), 371 (12), 369 (23), 367 (13), 290 (16), 288 (16), 277 (23), 275 (21), 267 (14), 265 (28), 263 (16), 254 (10), 252 (20), 250 (10), 218 (15), 217 (75), 216 (46), 215 (85), 214 (35), 213 (14), 203 (15), 201 (18), 197 (27), 187 (20), 185 (24), 181 (18), 169 (16), 168 (23), 152 (21), 139 (28), 135 (49) and 107 (32%).

Isovaleramide. T. setosus residue after chloroform extraction was kept in acetone for several months. The acetone extract was concentrated, and the residue was chromatographed on a column of Bio-Sil A. The column was eluted with chloroform (2000 ml), chloroform-acetone 10:1 (1400 ml), 5:1 (800 ml), and 1:1 (400 ml). The last fraction gave a crystalline product (13 mg from 1 kg annelid), which on sublimation *in vacuo* afforded isovaleramide: m.p. 132–134° (lit.[18] m.p. 137°); ν_{max} (KBr) 3360, 3190, 2957, 1660 and 1630 cm^{-1}; δ ($CDCl_3$) 1·02 (6H, d, J 6·5 Hz) and 2·06–2·13 (3H, m); *m/e* 101 (5), 86 (13), 69 (2), 59 (100), 44 (21), and 43 (12%).

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