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SOME SIMPLE ANTHELMINTICS

BY

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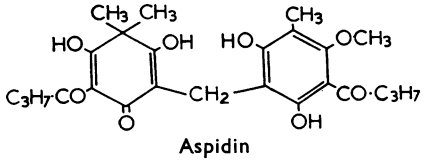
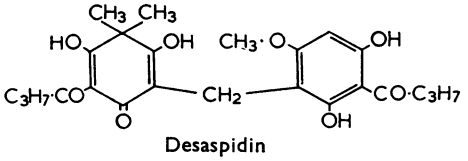
The substances mainly responsible for the anthelmintic activity of the rhizomes of male fern (*Dryopteris filix-mas*) and related plants were shown by Boehm early in the century to be complex phloroglucinol compounds, of which examples are flavaspidic acid and aspidin.

More recently the structures of related active compounds occurring in ferns have been elucidated by Penttilä & Sundman (1963 and earlier papers). Aspidin and desaspidin have been shown to be among the most active in the series (Blakemore, Bowden, Broadbent & Drysdale, 1964).



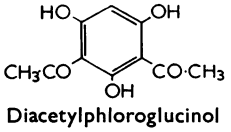
Aspidin



Desaspidin

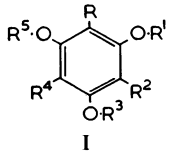
Although these compounds are effective against certain helminthic infections, they are not readily obtained in quantity from natural sources and their synthesis is not easy.

The object of the work was to find simpler phloroglucinol or related compounds with an anthelmintic action and which could be readily synthesized. During the preparation of substances related to desaspidin it was found that the simple compound diacetylphloroglucinol had appreciable activity *in vitro* against *Hymenolepis nana*.



Diacetylphloroglucinol

A series of related compounds, with structures I to VI, has been prepared in order to find the structural requirements for anthelmintic activity.



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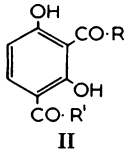

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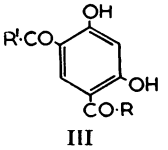

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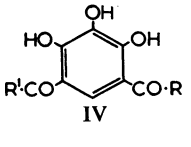
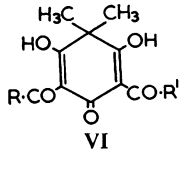


IV

V



VI

METHODS

Several known compounds were prepared in the following ways:

Acetylphloroglucinol (phloracetophenone; SK&F 90,531. I; $R=R^1=R^3=R^4=R^5=H$; $R^2=CO.CH_3$), by the method of Vogel (1959).

Diacetylfilicinic acid (SK&F 90,528. VI; $R=R^1=CH_3$) by the method of Hoefer & Reidl (1962).

The following compounds by the method of Campbell & Coppinger (1951):

1,3,5-*Triacetoxybenzene* (phloroglucinol triacetate; SK&F 90,532. I; $R=R^2=R^4=H$; $R^1=R^3=R^5=CO.CH_3$), melting point 104 to 106° C.

Diacetylphloroglucinol (SK&F 90,525. I; $R=R^1=R^3=R^5=H$; $R^2=R^4=CO.CH_3$), melting point 168° C. It appears likely that the compound with a melting point of 153° C and claimed by Israelstam (1943) to be diacetylphlorglucinol was, in fact, triacetylphloroglucinol.

Triacetylphloroglucinol (SK&F 90,533. I; $R=R^2=R^4=CO.CH_3$; $R^1=R^3=R^5=H$), melting point 152–154° C, was prepared by the method of Heller (1909).

The following compounds were prepared by the method of Dean & Robertson (1953):

2,4-*Diacetylphloroglucinol* 1-*methyl ether* (SK&F 90,548. I; $R=R^3=R^5=H$; $R^2=R^4=CO.CH_3$; $R^1=CH_3$), melting point 104° C.

2,4-*Diacetylphloroglucinol* 1,5-*dimethyl ether* (SK&F 90,543. I; $R=R^3=H$; $R^1=R^5=CH_3$; $R^2=R^4=CO.CH_3$), melting point 128–130° C.

2,4-*Diacetylphloroglucinol* 1,3,5-*trimethyl ether* (SK&F 90,547. I; $R=H$; $R^1=R^3=R^5=CH_3$; $R^2=R^4=CO.CH_3$), melting point 109–111° C.

1,3,5-*Triacetoxy*-2,4-*diacetylbenzene* (SK&F 90,651. I; $R=H$; $R^1=R^2=R^3=R^4=R^5=COCH_3$), melting point 92–93° C.

2,4-*Diacetyl*-1,3,5-*tribenzoyloxybenzene* (2,4-diacetylphloroglucinol 1,3,5-tribenzoate; SK&F 90,673. I; $R=H$; $R^2=R^4=CO.CH_3$; $R^1=R^3=R^5=CO.C_6H_5$), melting point 135–137° C.

4-*Butyrylphloroglucinol* 1-*methyl ether* (phlorobutyrophenone 4-methyl ether; SK&F 90,540. I; $R=R^1=R^3=R^4=H$; $R^2=CO.C_3H_7^n$; $R^5=CH_3$), melting point 123–125° C, was prepared by the method of Karrer (1919).

γ-*Methylvalerylphloroglucinol monohydrate* (SK&F 90,681. I; $R=R^1=R^3=R^4=R^5=H$; $R^2=CO.[CH_2]_2.CH(CH_3)_2$), melting point 101–103° C, was prepared by the method of Karrer & Rosenfeld (1921).

Valerylphloroglucinol monohydrate (SK&F 90,774. I; $R=R^1=R^3=R^4=R^5=H$; $R^2=CO.C_4H_9^n$), melting point 84–86° C, was prepared according to the procedure of Howells & Little (1932).

4-*Butyrylphloroglucinol* 1,3-*dimethyl ether* (2,4-dimethoxy-6-hydroxybutyrophenone; SK&F 90,524. I; $R=R^1=R^4=H$; $R^3=R^5=CH_3$; $R^2=CO.C_3H_7^n$), melting point 70° C, was prepared by the method of Canter, Curd & Robertson (1931).

2,4-*Diacetyl*-6-*methylphloroglucinol* (SK&F 90,536. I; $R=CH_3$; $R^1=R^3=R^5=H$; $R^2=R^4=CO.CH_3$) was prepared by the following method (compare Dean & Robertson, 1953). Methylphloroglucinol (10 g) in boron trifluoride–acetic acid complex (100 ml.) was heated on a boiling-water bath for 4 hr, and the resulting mixture was poured into sodium acetate solution (50 g of anhydrous sodium acetate in 350 ml. of water), whereupon a yellow solid separated. This was filtered off and recrystallized from methanol, yielding the product as colourless needles, melting point 165–167° C; yield 15 g (62.5% of theory).

The following ethers of 2,4-diacetyl-6-methylphloroglucinol were prepared by the method of Dean & Robertson (1953):

2,4-*Diacetyl*-6-*methylphloroglucinol* 1-*methyl ether* (SK&F 90,552. I; $R=R^1=CH_3$; $R^2=R^4=CO.CH_3$; $R^3=R^5=H$), melting point 95–97° C.

2,4-*Diacetyl*-6-*methylphloroglucinol* 1,3,5-*trimethyl ether* (SK&F 90,546. I; $R=R^1=R^3=R^5=CH_3$; $R^2=R^4=CO.CH_3$), melting point 66–67° C.

4,6-*Dipropionylpyrogallol* (SK&F 90,670. IV; $R=R^1=C_2H_5$), melting point 188° C, was prepared by the method of Israelstam (1943).

1,3-*Diacetyl*-2,4,5-*trihydroxybenzene* (SK&F 90,686. V; $R=R^1=CH_3$), melting point 186–187° C, was made by the procedure of Healey & Robinson (1934).

Resorcinol compounds

Israelstam (1943) has described the preparation of 2,4- and 4,6-dipropionylresorcinols by heating resorcinol with propionic anhydride in the presence of sulphuric acid as catalyst. The isomers were

separated by steam-distillation and sublimation *in vacuo*. We were unable to obtain a complete separation of isomers by these methods. Separation was achieved on a silica gel column, the purity of the fractions from the column containing the individual isomers being assayed by thin-layer chromatography on Kieselgel G, using benzene as a developing solvent. When sulphuric acid was used as a catalyst in the reaction, the 4,6-isomer was found to predominate, whereas the use of boron trifluoride as a catalyst gave mainly the 2,4-isomer. These methods were also used for the preparation of the dibutyryl- and divaleryl-resorcinols.

Preparation of 2,4-*dipropionylresorcinol* (SK&F 90,634-1. II; $R=R^1=C_2H_5$) *and* 4,6-*dipropionylresorcinol* (SK&F 90,650. III; $R=R^1=C_2H_5$)

To a mixture of resorcinol (5.5 g) and propionic anhydride (19.5 g) was added concentrated sulphuric acid (3.0 ml.). The mixture was heated to 130° C and held at that temperature for 15 min. The product was poured into water, the suspension filtered, and the residue dried to give the crude product (3.0 g).

The crude product was dissolved in benzene (5 ml.) and fractionated through a column of silica-gel (Light's 50–100 mesh, for chromatography), 2 cm in diameter and 145 cm long, made up in benzenel Fractions of approximately 50 ml. were collected and tested for purity by spotting on to plates of Kieselge. G of 250 μ thickness. The plates were developed in benzene at room temperature for 30 min, dried and sprayed with a 0.1% aqueous solution of Fast Blue Salt B (Merck). The isomers were revealed as red spots on a white background. The 2,4-dipropionylresorcinol had $R_F=0.4$ and the 4,6-isomer $R_F=0.196$ By these means, the pure isomers were collected from the column and recrystallized from absolute alcohol. 2,4-Dipropionylresorcinol melted at 85.5° C (50 mg) and 4,6-dipropionylresorcinol (0.73 g) at 128.5° C.

A mixture of resorcinol (5.5 g), propionic anhydride (13 g) and redistilled boron trifluoride etherate (50 ml.) was heated on a boiling-water bath for 2 hr. The hot solution was poured into 20% aqueous ethanol (100 ml.) containing sodium acetate (50 g). The ethereal layer was separated and treated with saturated aqueous bicarbonate solution (50 ml.) for 2 hr at room temperature. The ethereal layer was run off, dried (magnesium sulphate) and evaporated to give an oil which solidified (8.5 g). Part of this material was fractioned through a silica-gel column as in the previous experiment to give pure 2,4-dipropionylresorcinol (0.75 g) and pure 4,6-dipropionylresorcinol (0.16 g).

2,4-Dibutyrylresorcinol, 4,6-dibutyrylresorcinol and 4,6-divalerylresorcinol were prepared by similar procedures and are listed in the new compounds given below.

New compounds

4,6-*Dibutyrylpyrogallol* (SK&F 90,678. IV; $R=R^1=C_3H_7'$) was prepared from butyric anhydride and pyrogallol in the presence of sulphuric acid (compare Israelstam, 1943). It crystallized from glacial acetic acid and melted at 142–143.5° C. (Found: C, 63.0; H, 6.9. $C_{14}H_{18}O_5$ requires C, 63.1; H, 6.8%.)

4,6-*Dibutyrylresorcinol* (SK&F 90,658. III; $R=R^1=C_3H_7'$), melting point 64.5–65° C (from ethanol). (Found: C, 67.4; H, 7.1. $C_{14}H_{18}O_4$ requires C=67.2; H=7.25%.) R_F on thin-layer Kieselgel G plates=0.26.

2,4-*Dibutyrylresorcinol* (SK&F 90,671. II; $R=R^1=C_3H_7''$), melting point 29–30° C (from ethanol). (Found: C, 66.7; H, 7.1.) R_F on thin-layer Kieselgel G plates=0.38.

4,6-*Divalerylresorcinol* (SK&F 90,685. III; $R=R^1=C_4H_9''$). An oil, boiling point 127–130° C, 1.4×10^{-4} mm Hg pressure. (Found: C, 69.5; H, 8.1. $C_{16}H_{22}O_4$ requires C, 69.0; H, 8.0%.) R_F on thin-layer Kieselgel G plates=0.22.

Tributyrylphloroglucinol (SK&F 90,545. I; $R^1=R^3=R^5=H$; $R=R^2=R^4=CO.C_3H_7''$) was prepared by heating together anhydrous phloroglucinol (2.5 g), butyric anhydride (10 g) and concentrated sulphuric acid (1 ml.) at 130–135° C for 10 min. The product was poured into water, the mixture filtered and the dried residue crystallized from absolute alcohol to yield the product as needles, melting point 96–97.5° C (1.75 g). (Found: C, 63.95; H, 7.0; O, 28.8. $C_{18}H_{24}O_6$ requires C, 64.3; H, 7.2; O, 28.5%.)

Israelstam (1943), using propionic anhydride under similar conditions, claimed the formation of 2,4-dipropionylphloroglucinol with melting point 137–138° C. We found that this compound, prepared by the method below, melts at 152–154° C. In the light of the formation of 2,4,6-tributyrylphloroglucinol in the experiment described above it appears likely that the product obtained by Israelstam from propionic anhydride and phloroglucinol was 2,4,6-tripropionylphloroglucinol.

1,3,5-*Triacetoxy*-2,4-*dipropionylbenzene* (SK&F 90,700. I; R=H; $R^1=R^3=R^5=CO.CH_3$; $R^2=R^4=CO.C_2H_5$) was prepared in 70% yield from 2,4-dipropionylphloroglucinol by treatment with acetic anhydride in pyridine. The compound, crystallized from a mixture of ethyl acetate and light petroleum (boiling point 40–60° C), melted at 88° C. (Found: C, 59.3; H, 5.5; O, 35.1. $C_{16}H_{20}O_8$ required C, 59.4; H, 5.3; O, 35.1%.)

Preparation of 2,4-*diacylphloroglucinols where both acyl groups are equal*

These compounds were prepared from phloroglucinol and the requisite anhydride in the presence of boron trifluoride etherate. The following preparation of 2,4-divalerylphloroglucinol is a typical example of the method used.

2,4-*Divalerylphloroglucinol* (SK&F 90,590. I; $R=R^1=R^3=R^5=H$; $R^2=R^4=CO.C_4H_9^n$). Anhydrous phloroglucinol (6.3 g, 0.05 mole), valeric anhydride (18.6 g, 0.1 mole) and boron trifluoride etherate (50 ml.) were heated on a boiling-water bath for 4 hr. The clear red solution was poured into sodium acetate solution (50 g of sodium acetate trihydrate in 100 ml. of water) and the ether layer was separated. The aqueous portion was extracted with two 100-ml. portions of ether, and the combined ethereal extracts were treated with aqueous sodium bicarbonate solution to remove valeric acid, washed with water (100 ml.) and dried (magnesium sulphate). Removal of the solvent from the dried solution gave a red oil, which was then warmed under reduced pressure to remove the aliphatic ketones formed as by-products (boiling point about 50° C at 20 mm Hg pressure). The residue was crystallized (charcoal) several times from a mixture of ethyl acetate and light petroleum (boiling point 60–80° C) to yield 2,4-divalerylphloroglucinol as colourless needles, melting point 104–106° C. A further quantity of the product was obtained by dissolving the residue after crystallization in light petroleum (boiling point 60–80° C) and percolating the solution through alumina (Peter Spence, type H). Yield=50% theory. (Found: C, 65.4; H, 7.5; O, 27.4. $C_{16}H_{22}O_5$ requires C, 65.3; H, 7.5; O, 27.2%.)

A series of compounds obtained by similar procedures is tabulated below (Table 1).

Several trimethyl ethers of the diacylphloroglucinols were prepared by the general method typified below.

Preparation of 2,4-*dipropionylphloroglucinol* 1,3,5-*trimethyl ether* (SK&F 90,575. I; R=H; $R^1=R^3=R^5=CH_3$; $R^2=R^4=CO.C_2H_5$)

2,4-Dipropionylphloroglucinol (3 g) in dry acetone containing an excess of dimethyl sulphate (8 ml.) and potassium carbonate (12 g) was heated under reflux for 5 hr. The inorganic salts were filtered off and washed with acetone (50 ml.); the filtrate and washings were evaporated, and the resulting yellow oil was treated with sodium hydroxide solution. The mixture was extracted with ether (3 × 50 ml.), and the extracts were washed with water (1 × 50 ml.), dried (magnesium sulphate) and evaporated to dryness to give an oil which solidified on cooling in ice. The product (3 g) crystallized from light petroleum (boiling point 60–80° C) in large rhombs, melting point 95–97° C. (Found: C, 64.3; H, 7.2; OCH_3, 33.2. $C_{15}H_{20}O_5$ requires C, 64.1; H, 7.3; OCH_3, 33.1%.)

By similar procedures the following compounds were prepared:

2,4-*Dibutyrylphloroglucinol* 1,3,5-*trimethyl ether* (SK&F 90,568. I; R=H; $R^1=R^3=R^5=CH_3$; $R^2=R^4=CO.C_3H_7^n$), melting point 70–72° C, from light petroleum (boiling point 40–60° C). Found: C, 66.0; H, 7.7; O, 25.95; OCH_3, 30.0. $C_{17}H_{24}O_5$ requires C, 66.2; H, 7.85; O, 25.9; OCH_3, 30.2%.)

2,4-*Di-isobutyrylphloroglucinol* 1,3,5-*trimethyl ether* (SK&F 90,597. I; R=H; $R^1=R^3=R^5=CH_3$; $R^2=R^4=CO.CH(CH_3)_2$), melting point 115–116° C, from light petroleum (boiling point 60–80° C). (Found: C, 66.3; H, 7.8; O, 26.1; OCH_3, 30.1. $C_{17}H_{24}O_5$ requires C, 66.2; H, 7.85; O, 25.9; OCH_3, 30.2%.)

6-*Methyl*-2,4-*dipropionylphloroglucinol* 1,3,5-*trimethyl ether* (SK&F 90,582. I; $R=R^1=R^3=R^5=CH_3$; $R^2=R^4=CO.C_2H_5$), melting point 88–90° C, from light petroleum (boiling point 40–60° C). (Found: C, 65.3; H, 7.5; OCH_3, 31.3. $C_{16}H_{22}O_5$ requires C, 65.3; H, 7.5; OCH_3, 31.6%.)

Preparation of diacylphloroglucinols containing two different acyl groups

2-*Acetyl*-4-*propionylphloroglucinol* (SK&F 90,617. I; $R=R^1=R^3=R^5=H$; $R^2=CO.CH_3$; $R^4=CO.C_2H_5$). Anhydrous phloracetophenone (8.4 g, 0.05 mole) in propionic anhydride (6.5 g, 0.05 mole)

TABLE 1

PROPERTIES OF COMPOUNDS USED

B.p.=boiling point, m.p.=melting point in °C

SK&F No.	Compound, structure, yield	Crystallizing solvent, crystalline form, melting point	Formula	Analysis					
				Required			Found		
				C	H	O	C	H	O
90,567	2,4-Dipropionylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_2H_5) 60%	Ethyl acetate/light petroleum (b.p. 60–80°). Needles. M.p. 152–154°	$C_{12}H_{14}O_5$	60·5	5·9	33·6	60·4	5·7	33·8
90,562	2,4-Dibutyrylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_3H_{7n}) 60%	Ethyl acetate/light petroleum (b.p. 60–80°). Needles. M.p. 135–137°	$C_{14}H_{18}O_5$	63·1	6·8	30·0	63·4	6·6	30·0
90,569	2,4-Di-isobutyrylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_3H_{7i}) 71%	Light petroleum (b.p. 40-60°). Prisms. M.p. 128–130°	$C_{14}H_{18}O_5$	63·1	6·8	30·0	63·2	6·8	29·8
90,616	2,4-Dihexanoylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_5H_{11n}) 25%	Light petroleum (b.p. 60–80°). Needles. M.p. 97–98°	$C_{18}H_{26}O_5$	67·1	8·1	24·8	67·4	8·2	24·7
90,620	2,4-Diheptanoylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_6H_{13n}) 30%	Light petroleum (b.p. 60–80°). Needles. M.p. 96–98°	$C_{20}H_{30}O_5$	68·5	8·6	22·8	68·7	8·6	22·8
90,621	2,4-Di(γ-methylvaleryl)phloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.$[CH_2]_2$.$CH(CH_3)_2$) 34%	Light petroleum (b.p. 60–80°). Needles. M.p. 114–116°	$C_{18}H_{26}O_5$	67·1	8·1	24·8	67·0	8·1	24·9
90,656	2,4-Dioctanoylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_7H_{15n}) 32%	Light petroleum (b.p. 60–80°). Needles. M.p. 93–95°	$C_{22}H_{34}O_5$	69·8	9·05	21·1	70·0	9·1	21·0
90,657	2,4-Di-isovalerylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.CH_2.$CH(CH_3)_2$) 40%	Light petroleum (b.p. 40–60°). Plates. M.p. 113–114°	$C_{16}H_{22}O_5$	65·3	7·5	27·2	65·5	7·6	27·3
90,648	2,4-Dinonanoylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.C_8H_{17n}) 29%	Light petroleum (b.p. 40–60°). Waxy needles. M.p. 84–87°	$C_{24}H_{38}O_5$	70·9	9·4	19·7	70·9	9·4	19·85
90,665	2,4-Didecanoylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=CO.CH_9H_{19n}) 42%	Light petroleum (b.p. 40–60°). Needles. M.p. 80–82°	$C_{26}H_{42}O_5$	71·85	9·7	18·4	72·0	9·85	18·3
90,578	6-Methyl-2,4-dipropionylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=CO.C_2H_5) 59%	Ethyl acetate/light petroleum (b.p. 60–80°). Needles. M.p. 135–137°	$C_{13}H_{16}O_5$	61·9	6·4	31·7	61·9	6·4	31·6

Table I.—*continued*

SK&F No.	Compound, structure, yield	Crystallizing solvent, crystalline form, melting point	Formula	Analysis Required C	Required H	Required O	Found C	Found H	Found O
90,574	2,4-Dibutyryl-6-methylphloroglucinol (I: R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_3H_{7n}$) 50%	Ethyl acetate/light petroleum (b.p. 60–80°). Prisms. M.p. 120–121°	$C_{15}H_{20}O_5$	64·3	7·2	28·5	64·4	7·1	28·3
90,589	2,4-Di-isobutyryl-6-methylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.CH(CH_3)_2$) 78%	Light petroleum (b.p. 60–80°). Needles. M.p. 115–116°	$C_{15}H_{20}O_5$	64·3	7·2	28·5	64·4	7·1	28·3
90,592	6-Methyl-2,4-divalerylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_4H_{9n}$) 30%	Light petroleum (b.p. 60–80°). Needles. M.p. 98–100°	$C_{17}H_{24}O_5$	66·2	7·85	25·9	66·1	7·7	26·1
90,649	2,4-Di-isovaleryl-6-methylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.CH_2.CH(CH_3)_2$) 40%	Light petroleum (b.p. 60–80°). Plates. M.p. 123–125°	$C_{17}H_{24}O_5$	66·2	7·85	25·9	66·4	8·0	26·0
90,625	2,4-Dihexanoyl-6-methylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_5H_{11n}$) 53%	Light petroleum (b.p. 60–80°). Needles. M.p. 108–110°	$C_{19}H_{28}O_5$	67·8	8·4	23·8	67·73	8·2	23·8
90,770	6-Methyl-2,4-di(γ-methylvaleryl)phloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.[CH_2]_2.CH(CH_3)_2$) 35%	Light petroleum (b.p. 60–80°). Plates. M.p. 85–88°	$C_{19}H_{28}O_5$	67·8	8·4	23·8	67·7	8·4	23·8
90,629	2,4-Diheptanoyl-6-methylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_6H_{13n}$) 30%	Light petroleum (b.p. 60–80°). Needles. M.p. 108–110°	$C_{21}H_{32}O_5$	69·2	8·85	21·95	69·4	9·0	21·85
90,655	6-Methyl-2,4-dioctanoylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_7H_{15n}$) 55%	Light petroleum (b.p. 60–80°). Needles. M.p. 102–104°	$C_{23}H_{36}O_5$	70·4	9·2	20·4	70·4	9·3	20·5
90,666	6-Methyl-2,4-dinonanoylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_8H_{17n}$) 48%	Light petroleum (b.p. 60–80°). Needles. M.p. 100–102°	$C_{25}H_{40}O_5$	71·4	9·6	19·0	71·5	9·6	19·15
90,674	2,4-Didecanoyl-6-methylphloroglucinol (I; R=CH_3; R^1=R^3=R^5=H; R^2=R^4=$CO.C_9H_{19n}$) 42%	Benzene/light petroleum (b.p. 60–80°). Needles. M.p. 99–101°	$C_{27}H_{44}O_5$	72·3	9·9	17·8	72·3	10·0	17·7
90,642	2,4-Diphenylacetylphloroglucinol (I; R=R^1=R^3=R^5=H; R^2=R^4=$CO.CH_2.C_6H_5$) 31%	Benzene/light petroleum (b.p. 60–80°). Pale yellow platelets. M.p. 170–172°	$C_{22}H_{18}O_5$	72·9	5·0	22·1	73·0	5·2	21·6

and redistilled boron trifluoride etherate (40 ml.) were heated on a boiling-water bath for 2 hr. The pale red solution was poured into sodium acetate solution (50 g of sodium acetate trihydrate in 100 ml. of water). The ether was removed by warming on the water-bath, leaving an oil which solidified at 0° C. The crude product (11.7 g) crystallized as a hydrate from aqueous alcohol in the form of blades, melting point 103–105° C. The anhydrous form was obtained by crystallization from benzene, melting point 149–150.5° C. (Found: C, 59.0; H, 5.4; O, 35.55. $C_{11}H_{12}O_5$ requires C, 58.9; H, 5.4; O, 35.7%.)

The following compounds were prepared in a similar manner:

2-Acetyl-4-butyrylphloroglucinol (SK&F 90,599. I; $R=R^1=R^3=R^5=H$; $R^2=CO.CH_3$; $R^4=CO.C_3H_7^n$), melting point 95–97° C, as the monohydrate from aqueous alcohol. (Found: C, 56.7; H, 5.65; O, 37.3. $C_{12}H_{14}O_5,H_2O$ requires C, 56.7; H, 5.55; O, 37.8%.)

2-Acetyl-4-valerylphloroglucinol (SK&F 90,644. I; $R=R^1=R^3=R^5=H$; $R^2=CO.CH_3$; $R^4=CO.C_4H_9^n$), melting point 129–131° C, from aqueous alcohol. (Found: C, 61.9; H, 6.2; O, 31.65. $C_{13}H_{16}O_5$ requires C, 61.9; H, 6.4; O, 31.7%.)

Preparation of diacylphloroglucinols containing a halogen atom

2-Bromo-4,6-dibutyrylphloroglucinol (SK&F 90,717. I; $R=Br$; $R^1=R^3=R^5=H$; $R^2=R^4=CO.C_3H_7^n$) was prepared as follows: 2,4-dibutyrylphloroglucinol (5.08 g, 0.02 mole) in chloroform (75 ml.) was stirred at room temperature. Bromine (3.2 g, 0.02 mole) in chloroform (15 ml.) was added over 15 min and stirring continued for a further 15 min. The mixture was then washed several times with water and once with a saturated aqueous sodium bicarbonate solution (50 ml.). The chloroform layer was separated and dried (magnesium sulphate), and the solvent was removed to give a white solid (6.2 g) which crystallized from light petroleum (boiling point 60–80° C) as fine needles, melting point 103–104.5° C. Yield 90% of theory. (Found: C, 48.8; H, 4.9; Br, 23.1. $C_{14}H_{17}BrO$ requires C, 48.7; H, 5.0; Br, 23.15%.)

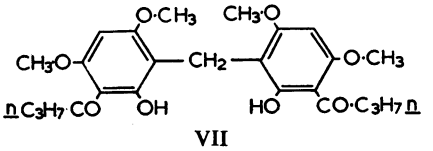
The following compound was prepared in a similar manner:

2-Bromo-4,6-divalerylphloroglucinol (SK&F 90,771. I; $R=Br$; $R^1=R^3=R^5=H$; $R^2=R^4=CO.C_4H_9^n$). Platelets, melting point 89–91° C, from light petroleum (boiling point 40–60° C). (Found: C, 51.6; H, 5.5; Br, 21.35; O, 21.5. $C_{16}H_{21}BrO_5$ requires C, 51.5; H, 5.4; Br, 21.4; O, 21.4%.)

Miscellaneous compounds

6-Methyl-2-phenylacetylphloroglucinol (SK&F 90,694. I; $R=CH_3$; $R^1=R^3=R^4=R^5=H$; $R^2=CO.CH_2.C_6H_5$) was prepared by heating together methylphloroglucinol (7.0 g, 0.05 mole) and phenylacetic anhydride (25.4 g, 0.1 mole) in boron trifluoride etherate (50 ml.) on a boiling-water bath for 4 hr. The red-brown mixture was poured into aqueous sodium acetate solution (50 g of sodium acetate trihydrate in 100 ml. of water) and the resulting mixture extracted with ether (3 × 100 ml.). The combined ethereal extracts were washed repeatedly with sodium bicarbonate solution, then with water and dried (magnesium sulphate). Removal of the solvent *in vacuo* gave a red oil which solidified on trituration with light petroleum (boiling point 60–80° C). Recrystallization from a mixture of benzene and light petroleum (boiling point 60–80° C) gave the product (4 g) as pale yellow plates, melting point 192–194° C (decomposition). (Found: C, 69.9; H, 5.6; O, 24.7. $C_{15}H_{14}O_4$ requires C, 69.75; H, 5.5; O, 24.8%.)

2,2′-Methylenebis(4-butyrylphloroglucinol 1,5-dimethyl ether) (SK&F 90,530. Structure VII).



VII

The compound was prepared by treating phlorobutyrophenone 2,4-dimethyl ether (1.1 g, 0.005 mole) in potassium hydroxide solution (0.56 g in 5.6 ml. of water) with formaldehyde (0.3 ml. of 40% aqueous solution) at 0° C and leaving at room temperature for 30 min. The mixture was acidified with dilute hydrochloric acid, and the precipitate was filtered off, dried and crystallized from light petroleum (boiling point 40–60° C) to give blades (1.0 g), melting point 67–69°. (Found: C, 65.0; H, 7.2. $C_{25}H_{32}O_8$ requires C, 65.2; H, 7.0%.)

Biological testing

The compounds were tested for activity against the dwarf tapeworm *Hymenolepis nana* by an *in vitro* method based on that of Sen & Hawking (1960) and by an *in vivo* method using mice infected with *H. nana*, based on that of Steward (1955). The details of both tests have been described elsewhere (Blakemore *et al.*, 1964).

In the *in vitro* tests worms were incubated for 24 hr at 37° C in a nutrient broth containing antibiotics. Various dilutions of the drug being tested were prepared in the broth and one worm was incubated with each dilution. In this way the minimum lethal concentration of each drug was determined.

In the *in vivo* tests, groups of eight to ten infected mice were treated by stomach tube with the drug under test at a dose of 400 mg/kg body weight. The drugs were administered in a 1% aqueous sodium glycocholate solution. In all experiments a " control " group of mice, equal in number to each of the treated groups, received the glycocholate solution alone. The results were assessed by comparing worm counts on the treated groups with worm counts on the control group. The worm counts, computed by Steward's (1955) method, are shown in Table 2. Moreover, we compared the number of worm-free mice in each of the treated groups with the control group, though these results are not shown in the Table.

Numbers in parentheses refer to SK&F code numbers, and activities refer to minimal lethal concentrations.

RESULTS AND DISCUSSION

The results of the *in vitro* and *in vivo* tests against *H. nana* are given in Table 2.

Relationship between anthelmintic activity and structure

Phloroglucinol with one acetyl group attached to the benzene ring (90,531) showed low *in vitro* activity ($>1 : 10^4$) against *H. nana* but the introduction of a second acetyl group giving diacetylphloroglucinol (90,525) raised the *in vitro* activity to $1 : 5\times10^5$. When a third acetyl group was introduced (90,533) the activity *in vitro* fell again to the figure of $1 : 10^4$.

Methylation of one or more of the hydroxyl groups in diacetylphloroglucinol (90,543; 90,547; 90,548) again led to compounds with a low order of activity. On the other hand, acetylation of the hydroxyl groups of diacetylphloroglucinol did not enhance the *in vitro* activity but did produce a compound (90,651) with some activity *in vivo*. 2,4-Diacetyl-6-methylphloroglucinol (90,536), although less active in the *in vitro* test, showed moderate activity (61%) *in vivo*.

Lack of activity in related compounds, namely the diacylresorcinols (structures II and III), diacylpyrogallols (structure IV), diacylhydroxyquinols (structure V), and diacetylfilicinic acid (structure VI), showed the basic requirements for activity to be a phloroglucinol nucleus with two acyl groups attached.

It was expected that lengthening of the acyl groups would cause a rise in the *in vitro* anthelmintic activity and this proved to be so. Introduction of two butyryl groups into the phloroglucinol nucleus produced a compound (90,562) having activity *in vitro* at a dilution of $1 : 10^6$ and considerable activity *in vivo* (89%).

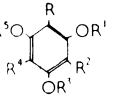
The corresponding compound with a halogen atom in the phloroglucinol nucleus (90,717) was less active *in vivo* (40%). So, too, was the compound, di-isobutyrylphloroglucinol (90,569) (62%), where the straight chains were replaced by branched chains containing the same number of carbon atoms.

Continuation of the study of the series showed that divalerylphloroglucinol (90,590) had high activity *in vitro* ($1 : 2\times10^7$) and *in vivo* (99%). Less activity was found in the

TABLE 2

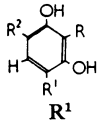
ACTIVITY OF THE COMPOUNDS USED

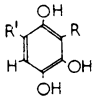
MLC = Minimal lethal concentration

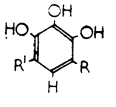
Compounds of the type 

SK&F No.	R	R^1	R^2	R^3	R^4	R^5	Activity against *H. nana* *In vitro* MLC	Activity against *H. nana* *In vivo* Anthelmintic activity (%)
90,524	H	H	$CO.C_3H_7''$	CH_3	H	CH_3	$>1:10^4$	0
90,525	H	H	$CO.CH_3$	H	$CH_3.CO$	H	$1:5\times10^5$	0
90,531	H	H	$CO.CH_3$	H	H	H	$>1:10^4$	0
90,532	H	$CO.CH_3$	H	$CO.CH_3$	H	$CH_3.CO$	$>1:10^4$	0
90,533	$CO.CH_3$	H	$CO.CH_3$	H	$CH_3.CO$	H	$1:10^4$	0
90,536	CH_3	H	$CO.CH_3$	H	$CH_3.CO$	H	$1:10^5$	61
90,540	H	H	$CO.C_3H_7''$	H	H	CH_3	$1:5\times10^5$	0
90,543	H	CH_3	$CO.CH_3$	H	$CH_3.CO$	CH_3	$>1:10^4$	0
90,545	$CO.C_3H_7''$	H	$CO.C_3H_7''$	H	$C_3H_7''.CO$	H	$>1:10^4$	17
90,546	CH_3	CH_3	$CO.CH_3$	CH_3	$CH_3.CO$	CH_3	$>1:10^4$	0
90,547	H	CH_3	$CO.CH_3$	CH_3	$CH_3.CO$	CH_3	$>1:10^4$	0
90,548	H	CH_3	$CO.CH_3$	H	$CH_3.CO$	H	$1:10^4$	0
90,552	CH_3	H	$CO.CH_3$	H	$CH_3.CO$	CH_3	$>1:10^4$	0
90,562	H	H	$CO.C_3H_7''$	H	$C_3H_7''.CO$	H	$1:10^6$	89
90,568	H	CH_3	$CO.C_3H_7''$	CH_3	$C_3H_7''.CO$	CH_3	$>1:10^4$	14
90,569	H	H	$CO.CH(CH_3)_2$	H	$(CH_3)_2CH.CO$	H	$1:10^6$	62
90,575	H	CH_3	$CO.C_2H_5$	CH_3	$C_2H_5.CO$	CH_3	$>1:10^4$	0
90,578	CH_3	H	$CO.C_2H_5$	H	$C_2H_5.CO$	H	$1:5\times10^5$	50
90,582	CH_3	CH_3	$CO.C_2H_5$	CH_3	$C_2H_5.CO$	CH_3	$1:10^4$	0
90,589	CH_3	H	$CO.CH(CH_3)_2$	H	$(CH_3)_2CH.CO$	H	$1:5\times10^5$	100
90,590	H	H	$CO.C_4H_9''$	H	$C_4H_9''.CO$	H	$1:2\times10^7$	99
90,592	CH_3	H	$CO.C_4H_9''$	H	$C_4H_9''.CO$	H	$1:10^7$	99
90,597	H	CH_3	$CO.CH(CH_3)_2$	CH_3	$(CH_3)_2CH.CO$	CH_3	$1:10^4$	14
90,599	H	H	$CO.C_3H_7''$	H	$CH_3.CO$	H	$1:10^6$	87
90,616	H	H	$CO.C_5H_{11}''$	H	$C_5H_{11}''.CO$	H	$1:2\times10^7$	100
90,617	H	H	$CO.C_2H_5$	H	$CH_3.CO$	H	$1:5\times10^5$	14
90,620	H	H	$CO.C_6H_{13}''$	H	$C_6H_{13}''.CO$	H	$1:10^7$	74
90,621	H	H	$CO.[CH_2]_2.CH(CH_3)_2$	H	$(CH_3)_2CH.[CH_2]_2.CO$	H	$1:2\times10^7$	99
90,625	CH_3	H	$CO.C_5H_{11}''$	H	$C_5H_{11}''.CO$	H	$1:10^7$	98
90,629	CH_3	H	$CO.C_6H_{13}''$	H	$C_6H_{13}''.CO$	H	$1:10^7$	99
90,642	H	H	$CO.CH_2.C_6H_5$	H	$C_6H_5.CH_2.CO$	H	$1:10^6$	13
90,644	H	H	$CO.C_4H_9''$	H	$CH_3.CO$	H	$1:10^6$	45
90,648	H	H	$CO.C_8H_{17}''$	H	$C_8H_{17}''.CO$	H	$1:10^6$	90
90,649	CH_3	H	$CO.CH_2.CH(CH_3)_2$	H	$(CH_3)_2CH.CH_2.CO$	H	$1:10^6$	90
90,651	H	$CO.CH_3$	$CO.CH_3$	$CO.CH_3$	$CH_3.CO$	$CH_3.CO$	$1:5\times10^5$	32
90,655	CH_3	H	$CO.C_7H_{15}''$	H	$C_7H_{15}''.CO$	H	$1:5\times10^6$	94
90,656	H	H	$CO.C_7H_{15}''$	H	$C_7H_{15}''.CO$	H	$1:10^6$	92
90,657	H	H	$CO.CH_2.CH(CH_3)_2$	H	$(CH_3)_2CH.CH_2.CO$	H	$1:5\times10^6$	83
90,665	H	H	$CO.C_9H_{19}''$	H	$C_9H_{19}''.CO$	H	$1:10^5$	20
90,666	CH_3	H	$CO.C_8H_{17}''$	H	$C_8H_{17}''.CO$	H	$1:10^6$	31
90,673	H	$CO.C_6H_5$	$CO.CH_3$	$CO.C_6H_5$	$CH_3.CO$	$C_6H_5.CO$	$>1:10^4$	0
90,681	H	H	$CO.[CH_2]_2.CH(CH_3)_2$	H	H	H	$1:10^4$	0
90,694	CH_3	H	$CO.CH_2.C_6H_5$	H	H	H	$1:10^4$	0
90,700	H	$CO.CH_3$	$CO.C_2H_5$	CH_3CO	$C_2H_5.CO$	$CH_3.CO$	$1:10^6$	0
90,717	Br	H	$CO.C_3H_7''$	H	$C_3H_7''.CO$	H	$1:5\times10^5$	40
90,770	CH_3	H	$CO.[CH_2]_2.CH(CH_3)_2$	H	$(CH_3)_2CH.[CH_2]_2.CO$	H	$1:5\times10^7$	98
90,771	Br	H	$CO.C_4H_9''$	H	$C_4H_9''.CO$	H	$1:10^6$	63
90,774	H	H	$CO.C_4H_9''$	H	H	H	$1:10^4$	0

Table 2.—*continued*

SK&F No.	Compounds of the type  R	R^1	R^2	Activity against *H. nana* *In vitro* MLC	Activity against *H. nana* *In vivo* Anthelmintic activity (%)
90,634–1	$CO.C_2H_5$	$CO.C_2H_5$	H	$1:10^4$	0
90,650	H	$CO.C_2H_5$	$C_2H_5.CO$	$>1:10^4$	0
90,658	H	$CO.C_3H_7{}^n$	$C_3H_7{}^n.CO$	$1:10^4$	0
90,671	$CO.C_3H_7{}^n$	$CO.C_3H_7{}^n$	H	$1:10^4$	0
90,685	H	$CO.C_4H_9{}^n$	$C_4H_9{}^n.CO$	$>1:10^4$	0

SK&F No.	Compound of the type  R	R^1		
90,686	$CO.CH_3$	$CH_3.CO$	$1:10^4$	0

SK&F No.	Compounds of the type  R	R^1		
90,670	$CO.C_2H_5$	$C_2H_5.CO$	$>1:10^4$	13
90,678	$CO.C_3H_7{}^n$	$C_3H_7{}^n.CO$	$>1:10^4$	0

Miscellaneous compounds

SK&F No.			
90,528	Diacetylfilicinic acid (VI)	$1:10^4$	0
90,530	Compound VII	$1:10^4$	0

corresponding branched-chain member (90,657), which was active *in vitro* in a dilution of $1:5\times10^6$ and which *in vivo* gave a clearance of 83% of the parasites.

Again, introduction of a halogen atom into the nucleus (90,771) gave a compound with reduced activity ($1:10^6$ *in vitro* and 63% *in vivo*) but substitution of a methyl group for the halogen atom (90,592) yielded a very active compound giving 99% clearance *in vivo*. In the corresponding compound with a methyl group in the nucleus and two branched valeryl chains (90,649), the *in vivo* activity fell to 90%. High activity was retained when the acyl groups were lengthened; dihexanoylphloroglucinol (90,616) was active *in vitro* at a dilution of $1:2\times10^7$ and was 100% effective *in vivo*, whilst the branched-chain homologue (90,621) was almost as active. Introduction of a methyl group into the phloroglucinol nucleus of the latter produced a compound (90,770) exceedingly active *in vitro* ($1:5\times10^7$).

Further lengthening of the acyl groups (90,629) led to a reduction of *in vitro* activity although the *in vivo* activity remained high. The trend to lower activity was more easily

discernible in the higher homologues 90,656 (1 : 10^6; 92%), 90,648 (1 : 10^6; 90%), 90,665 (1 : 10^5; 20%).

In vivo activity against *H. nana* in the straight-chain diacylphloroglucinols appears to reach a maximum when the acyl groups each contain about six carbon atoms, although there was a spread of high activity from C_4 to C_9.

Karrer & Rosenfeld (1921) prepared a series of monoacylphloroglucinols which were tested for anthelmintic activity. In this series the most active compound was γ-methylvalerylphloroglucinol (90,681). We have found this compound to possess low activity in the *in vitro* test against *H. nana* and to be devoid of activity *in vivo*.

We prepared a number of diacylphloroglucinols where the two acyl groups differed, but these were found to be less active than the symmetrical molecules. Introduction of aromatic, in place of aliphatic, acyl groups (90,642; 90,694) produced compounds of low activity.

SUMMARY

1. The problem was to evolve readily synthesized compounds with anthelmintic activity, related to the complex active principles present in male fern and other plants.

2. Derivatives of resorcinol, pyrogallol, hydroxyquinol, filicinic acid and phloroglucinol were prepared and tested *in vitro* against *Hymenolepis nana* and against the parasite in mice.

3. A series of phloroglucinol compounds had high *in vitro* and *in vivo* activities against *H. nana*. The main structural requirement for high activity was a phloroglucinol nucleus with two acyl groups attached.

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