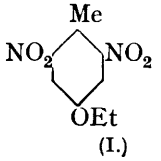
200. *Constituents of* Filix Mas. *Part I. Aspidinol.*

By Alexander Robertson and William F. Sandrock.

The extract of the dried rhizome of *Aspidium* (*Dryopteris*) *filix-mas* (or of *A. marginalis*) constitutes an important and reliable anthelmintic drug, *filix mas*, or oil of male fern, having a specific action on the various species of tapeworm. Our knowledge of the chemical constituents of this drug is due largely to Boehm, Kraft, Dacomono, and Schiff (for the main references, see Czapek, " Biochemie der Pflanzen," 1925, vol. III, pp. 565—567) and, though much work has been done, the chemical nature of the different compounds, aspidinol, filic acid, aspidin, albaspidin, flavaspidic acid, and filmaron, which appear to be derivatives of *C*-methyl- and of *gem*-dimethyl-phloroglucinol, is still not clear. The structural formulæ which have been ascribed to these substances depend on the results of analytical investigations and in the present series of memoirs it is intended to supply complementary evidence mainly by means of synthetical experiments.

Aspidinol, the simplest phenolic constituent of the drug and an integral part of the aspidin molecule, was first isolated in 1897 by Boehm (*Arch. exp. Path. Pharm.*, **38**, 35), who subsequently showed that the compound was a monomethyl ether of *C*-methylphloro-*n*-butyrophenone (VII or VIII); on the basis of indirect evidence he finally adopted formula (VII) for the ketone (*Annalen*, 1901, **318**, 245; 1903, **329**, 269). By the condensation of *C*-methylphloroglucinol β-methyl ether and *n*-butyronitrile Karrer and Widmer (*Helv. Chim. Acta*, 1920, **3**, 392) obtained two isomeric ketones, one of which was identical with Boehm's aspidinol. Accordingly, in view of the absence of direct experimental evidence regarding the orientation of aspidinol, the experiments now described were undertaken.

On repeating the condensation of *C*-methylphloroglucinol β-methyl ether and *n*-butyronitrile we always obtained a good yield of aspidinol accompanied by only a trace of Karrer and Widmer's *ψ*-aspidinol (less than 1% of the crude product). Karrer and Widmer have remarked, however, that the proportion of the isomerides formed varies to some extent with the experimental conditions. On the other hand, when *n*-butyronitrile is replaced by acetonitrile, Curd and Robertson (unpublished work) have found that only 2 : 6-dihydroxy-4-methoxy-3-methylacetophenone is formed. The latter authors (this vol., pp. **437**, **714**) have also shown that in general the application of the Gattermann and Hoesch (with acetonitrile) reactions to the α-dimethyl ether of *C*-methylphloroglucinol yields only the respective *o*-hydroxycarbonyl compounds. These results, together with the fact that aspidinol is almost insoluble in dilute aqueous sodium carbonate, led us to believe that the ketone is represented by formula (VII). Further, on the basis of the numerous alkylation experiments on carbonyl derivatives of *C*-methylphloroglucinol described by Curd and Robertson (*loc. cit.*) we considered it likely that on ethylation by means of ethyl iodide and potassium carbonate in boiling acetone aspidinol would give rise to a monoethyl ether only (IV). In agreement with these conclusions the orientation of aspidinol was established in the following manner.



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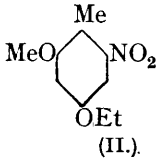

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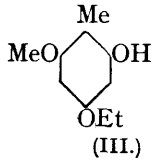

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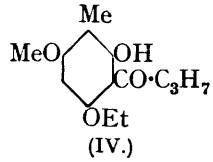

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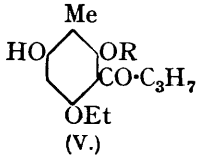

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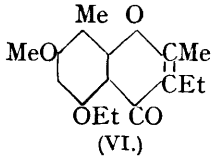

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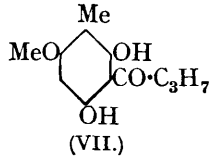

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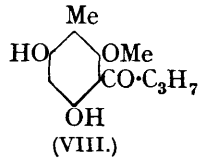

(VIII.)

6-*Methoxy*-4-*ethoxy*-o-*cresol* (III) was prepared from 2 : 6-dinitro-*p*-cresol by way of

the stages (I) and (II) and on condensation with *n*-butyronitrile according to the method of Hoesch gave rise to the *ketone* (IV) along with a small amount of the *isomeride* (V, R = Me). The former compound, which gave a ferric chloride reaction, was identical with the monoethyl ether of aspidinol and on ring closure with acetic anhydride and sodium acetate formed the *chromone* (VI). The orientation of monoethylaspidinol (IV) is thus clearly established and accordingly aspidinol must be represented by formula (VII).

Though the melting point originally given for the natural ketone by Boehm was 143° (identical with that of the synthetic compound), in a later communication (1901, *loc. cit.*) this author states that by repeated crystallisation from benzene a specimen was obtained melting at 156—161°, and that this melting point was considerably depressed by traces of impurities. From a comparison of two of Boehm's specimens, m. p. 140° and m. p. 145—156°, with their synthetic ketone, m. p. 140—141°, Karrer and Widmer concluded that the substances were identical. We were able, through the kindness of Professor O. Gros of Leipzig, to examine three small specimens of natural aspidinol, (A), (B), and (C), isolated by Boehm, and have confirmed the observations of Karrer and Widmer. In addition we found that on ethylation (B) and (C) gave a monoethyl ether identical with the ether derived from synthetic aspidinol and with the *o*-hydroxy-ketone obtained from (III) by the Hoesch reaction. After exhaustive purification, the melting point of our synthetic aspidinol remained constant at 143°. The natural specimen (A) having the higher melting point was too small to permit a detailed examination.

The only feasible direct method for establishing the relative positions of the hydroxyl and carbonyl groups in ketones of the type (IV) appeared to be the conversion of the latter into the corresponding chromones with acetic anhydride and sodium acetate. At first considerable difficulty was experienced in effecting this ring closure. Under the usual conditions, only the acetates of the ketones are formed, and on this account the conversion of 2-*hydroxy*-4 : 6-*dimethoxy*- and 2-*hydroxy*-4 : 6-*diethoxy*-3-*methyl*-n-*butyrophenone* into 5 : 7-*dimethoxy*- and 5 : 7-*diethoxy*-2 : 8-*dimethyl*-3-*ethylchromone* respectively was studied ; the orientation of the former ketone follows from its preparation by the general method of Curd and Robertson (*loc. cit.*).

The Reactivity of some C-*Methylphenolic Ethers.*

St. Pfau (*Helv. Chim. Acta*, 1928, **11**, 864) observed that contrary to expectation the application of the Gattermann reaction to β-orcinol monomethyl ether gave a product consisting mainly of the *o*-hydroxy-aldehyde and, from a comparison with the behaviour of orcinol monomethyl ether, suggested that the *C*-methyl group in the *o*-position to the methoxyl and hydroxyl groups was responsible for this result. The behaviour of 6-methoxy-*o*-cresol (Jones and Robertson, J., 1932, 1689) and of *C*-methylphloroglucinol β-monomethyl and α-dimethyl ethers (Curd and Robertson, *loc. cit.*) in yielding only the respective *o*-hydroxy-aldehydes amply supports the conclusion of St. Pfau. In addition, the work of Jones and Robertson (*loc. cit.*) and of Curd and Robertson (*loc. cit.*) and the experiments now described show that with these phenols the Hoesch reaction takes the same course (compare Baker and Robinson, J., 1929, 155 ; Robertson and co-workers, J., 1930, 21 ; 1931, 1245).

Experimental.

2 : 6-*Dinitro*-4-*ethoxytoluene* (I).—2 : 6-Dinitro-*p*-cresol (Curd and Robertson, *loc. cit.*) (20 g.) was ethylated with ethyl iodide (20 c.c.) and potassium carbonate (20 g.) in boiling acetone (60 c.c.) during 6 hours. After isolation in the usual manner, the *ether* crystallised from light petroleum (b. p. 60—80°) in pale yellow prisms and then from aqueous alcohol in straw-coloured laminæ, m. p. 108—109° (Found : C, 47·8; H, 4·7. $C_9H_{10}O_5N_2$ requires C, 47·8; H, 4·4%).

The use of ethyl sulphate gave unsatisfactory yields.

6-*Nitro*-4-*ethoxy*-o-*toluidine*.—To a solution of the foregoing ether (11·3 g.) in alcohol (75 c.c.), stannous chloride (33·8 g.), dissolved in alcohol (100 c.c.) saturated with dry hydrogen chloride, was added in 6 portions during ½ hour. The mixture was heated on the steam-bath for 2 hours, the greater part of the alcohol distilled, water (700 c.c.) added, the tin salt decomposed by 30% aqueous potassium hydroxide (500 c.c.), and the precipitate collected, washed, dried, and

repeatedly extracted with boiling light petroleum (b. p. 60—80°). On cooling, the extracts deposited the *amine* (8 g.) in orange needles which in contact with the solvent rapidly changed to irregular plates, m. p. 89—90° after being thrice recrystallised (Found : N, 14·5. $C_9H_{12}O_3N_2$ requires N, 14·3%). The *acetyl* derivative separated from aqueous alcohol in colourless needles, m. p. 192—193° (Found : N, 11·9. $C_{11}H_{14}O_4N_2$ requires N, 11·8%).

This nitro-amine (15 g.), m. p. and mixed m. p. 89—90°, was also obtained by the partial reduction of the dinitro-compound (22·7 g.) in warm alcohol (85 c.c.) with aqueous ammonia (13 c.c., *d* 0·88) and an excess of hydrogen sulphide during 2 hours.

6-*Nitro*-4-*ethoxy*-o-*cresol*.—The preceding nitro-amine (15 g.) was dissolved in water (225 c.c.) and sulphuric acid (45 c.c.) and diazotised at below 0° (5·3 g. of sodium nitrite in 30 c.c. of water). ½ Hour later the unchanged nitrous acid (trace) was decomposed with urea, and the diazo-solution gradually added to boiling 20% sulphuric acid (1 l.). After cooling, the solid (14·5 g.) was collected, washed, dried, and extracted with boiling light petroleum. The *nitro-cresol* (10·5 g.) separated from the extract in clusters of yellow needles, m. p. 117—118° after recrystallisation (Found in material dried in a high vacuum at 70° : C, 54·8; H, 5·9. $C_9H_{11}O_4N$ requires C, 54·8; H, 5·6%). The compound is soluble in benzene, alcohol, and ether.

Ethylation of this phenol (10 g.) with ethyl iodide (10 c.c.) and potassium carbonate (20 g.) in boiling acetone (25 c.c.) during 4 hours gave 2-*nitro*-4 : 6-*diethoxytoluene*, which separated from aqueous alcohol in slender yellow prisms, m. p. 103° (Found : C, 58·7; H, 6·8. $C_{11}H_{15}O_4N$ requires C, 58·7; H, 6·8%).

4 : 6-*Diethoxy*-o-*toluidine*.—The above nitro-ether (7 g.) was reduced (7 hours) in boiling 50% alcohol (100 c.c.) with sodium sulphide (35 g.), water (excess) added, and the amine isolated in ether and converted into the sulphate, which formed needles from warm water. The base did not solidify. The *acetyl* derivative separated from dilute alcohol and then from light petroleum–ethyl acetate in needles, m. p. 134—135° (Found : N, 6·0. $C_{13}H_{19}O_3N$ requires N, 5·9%).

C-*Methylphloroglucinol* α-*Diethyl Ether*.—The preceding sulphate (7 g.; water, 75 c.c.; sulphuric acid, 10 c.c.) was diazotised below 0° (sodium nitrite, 1·85 g.; water, 10 c.c.) and after 1½ hours the filtered solution was gradually added to boiling 18% sulphuric acid. *Methylphloroglucinol α-diethyl ether* was isolated in ether, distilled under reduced pressure, and crystallised from light petroleum (b. p. 60—80°), forming silky needles, m. p. 64—65°, readily soluble in alcohol or ether and sparingly soluble in hot water (Found : C, 67·1; H, 8·3. $C_{11}H_{16}O_3$ requires C, 67·4; H, 8·2%). The compound does not give a ferric chloride reaction.

Condensation of Methylphloroglucinol α-Diethyl Ether and n-*Butyronitrile*.—The diethyl ether (3·7 g.) was condensed with the nitrile (1·8 g.) in ether (50 c.c.) by means of zinc chloride (2 g.) and excess of hydrogen chloride, 36 hours later the resulting mixture of ketimines was collected, washed with ether, and boiled with water (70 c.c.) for ½ hour, and next day the product was extracted with cold 1% aqueous sodium hydroxide for 4 hours, washed with water, dried, and crystallised from light petroleum. 2-*Hydroxy*-4 : 6-*diethoxy*-3-*methyl*-n-*butyrophenone* formed colourless, elongated, rectangular plates (2·3 g.), m. p. 103·5—104·5°, and gave a brown coloration with alcoholic ferric chloride which faded on the addition of water (Found : C, 67·5; H, 8·4. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%). Treatment of the ketone (0·5 g.) with acetic anhydride (15 c.c.) and pyridine (2 c.c.) at 37° for 2 days gave the *acetate*, which separated from ligroin in rectangular prisms, m. p. 82—83° (Found : C, 66·2; H, 7·9. $C_{17}H_{24}O_5$ requires C, 66·2; H, 7·9%). This derivative did not give a ferric chloride reaction.

The sodium hydroxide extract on acidification gave a dark brown precipitate, consisting mainly of 4-*hydroxy*-2 : 6-*diethoxy*-3-*methyl*-n-*butyrophenone* (V; R = Et). This was ground with sodium carbonate solution (20 c.c.) and washed with water and then separated from warm dilute alcohol (charcoal) as an oil which gradually formed colourless elongated plates. Twice recrystallised from light petroleum (b. p. 60—80°), it was finally obtained in clusters of needles (0·25 g.), m. p. 68°, and did not give a ferric chloride reaction (Found : C, 67·3; H, 8·2%).

5 : 7-*Diethoxy*-2 : 8-*dimethyl*-3-*ethylchromone*.—A mixture of 2-hydroxy-4 : 6-diethoxy-3-methyl-*n*-butyrophenone (1 g.), acetic anhydride (15 c.c.), and sodium acetate (2 g.) was maintained at 230—240° for 16 hours and poured into water (150 c.c.). Two days later the product was isolated with ether and crystallised from ligroin, forming rosettes of short brown prisms. A solution of this material in warm alcohol (25 c.c.) was decolorised with charcoal and, after evaporation of the solvent, the residual *chromone* separated from light petroleum (b. p. 60—80°) in colourless pointed leaflets, m. p. 140—141° (Found : C, 70·5; H, 7·6. $C_{17}H_{22}O_4$ requires C, 70·3; H, 7·6%). The pale yellow solution of the compound in concentrated sulphuric acid exhibits a faint green fluorescence.

6-*Methoxy*-4-*ethoxy*-o-*toluidine*.—Methylation of 6-nitro-4-ethoxy-*o*-cresol (15 g.) with methyl sulphate and 15% aqueous potassium hydroxide gave the *methyl* ether (II), which crystallised from dilute alcohol in pale yellow needles (15 g.), m. p. 89—90° (Found : N, 6·9. $C_{10}H_{13}O_4N$ requires N, 6·6%). This nitro-ether (15 g.) was reduced by boiling with sodium sulphide (35 g.) in 50% alcohol (200 c.c.) for 8 hours, and the resulting base (oil) converted into the sulphate (13·5 g.). The *acetyl* derivative separated from dilute alcohol in needles, m. p. 132—133° (Found : N, 6·5. $C_{12}H_{17}O_3N$ requires N, 6·3%).

6-*Methoxy*-4-*ethoxy*-o-*cresol* (III).—This *phenol* was prepared from the aforementioned amine by the method used in preparing methylphloroglucinol α-diethyl ether and separated as a *hydrate* from moist light petroleum in clusters of colourless needles, m. p. 52° (Found in material dried over phosphoric oxide in a vacuum desiccator : C, 59·8; H, 8·2. $C_{10}H_{14}O_3,H_2O$ requires C, 60·0; H, 8·0%). A specimen sublimed in a vacuum at 100° had m. p. 59—60° (Found : C, 65·9; H, 7·7. $C_{10}H_{14}O_3$ requires C, 65·9; H, 7·7%).

***Condensation of* 6-*Methoxy*-4-*ethoxy*-o-*cresol* and n-*Butyronitrile*.**—The anhydrous phenol (6 g.) was condensed with *n*-butyronitrile (3·2 g.) in ether (100 c.c.) by means of zinc chloride (4 g.) and excess of hydrogen chloride and 48 hours later the resulting solid was hydrolysed by boiling with water (100 c.c.) for 1 hour. Next day the product was collected, extracted with 1·5% aqueous sodium hydroxide (70 c.c.), washed with water, dried, and crystallised from light petroleum. Thus obtained, 2-*hydroxy*-4-*methoxy*-6-*ethoxy*-3-*methyl*-n-*butyrophenone* (IV) formed colourless, elongated, rectangular plates (4·1 g.), m. p. 114—115° (Found : C, 66·6; H, 8·0. $C_{14}H_{20}O_4$ requires C, 66·7; H, 7·9%). Treatment of this ketone with acetic anhydride and pyridine at 100° for 4 hours gave the *acetate*, which separated from light petroleum in rod-like prisms, m. p. 72—73° (ferric chloride reaction negative) (Found : C, 65·3; H, 7·6. $C_{16}H_{22}O_5$ requires C, 65·3; H, 7·5%).

On acidification the dilute aqueous alkaline extract of the crude product deposited an oil which slowly crystallised. A solution of this material in 1% aqueous sodium hydroxide was filtered from a trace of the *o*-hydroxy-ketone and acidified with acetic acid, yielding 4-*hydroxy*-2-*methoxy*-6-*ethoxy*-3-*methyl*-n-*butyrophenone* (V, R = Me), which separated from light petroleum (b. p. 60—80°) in small colourless needles (0·3 g.), m. p. 59°, and did not give a ferric chloride reaction (Found : C, 66·2; H, 7·7%).

7-*Methoxy*-5-*ethoxy*-2 : 8-*dimethyl*-3-*ethylchromone* (VI).—2-Hydroxy-4-methoxy-6-ethoxy-3-methyl-*n*-butyrophenone (1·4 g.) was heated with acetic anhydride (20 c.c.) and sodium acetate (4 g.) at 240—245° for 16 hours. On isolation with ether the *chromone* crystallised from dilute alcohol (charcoal) and then from ligroin in almost colourless, fern-like clusters of needles, m. p. 132—133° (Found : C, 69·5; H, 7·1. $C_{16}H_{20}O_4$ requires C, 69·6; H, 7·3%).

Aspidinol (VII).—Hydrogen chloride was led into a mixture of methylphloroglucinol β-methyl ether (Herzig and Wenzel, *Monatsh.*, 1902, **23**, 100) (3·1 g.), *n*-butyronitrile (2 c.c.), zinc chloride (1·5 g.), and ether (25 c.c.) for 8 hours. More ether (20 c.c.) was added and the product which had separated as a brown oil then crystallised in the course of 48 hours. It was washed with ether and boiled with water (150 c.c.) for 15 minutes and the resulting solid was triturated with 10% sodium carbonate solution (50 c.c.) for 9 hours. The insoluble crude aspidinol was collected, washed, dried, and crystallised from xylene and then from benzene, forming pale yellow, slender needles which in contact with the solvent quickly changed to elongated rectangular plates (2·9 g.), m. p. 143°; setting point, 139—140°. Further purification of the ketone, *e.g.*, by recrystallisation from dilute alcohol and then 8 times from benzene, followed by fractional sublimation in a high vacuum (oil-bath at 210°), failed to raise the m. p.; on sublimation it formed lustrous yellow plates. The *diacetate*, obtained by heating aspidinol (0·4 g.) with acetic anhydride (5 c.c.) and pyridine (2 c.c.) on the steam-bath for 1 hour, separated from ligroin in elongated prisms, m. p. 68° (Found : C, 62·5; H, 6·6. $C_{16}H_{20}O_6$ requires C, 62·3; H, 6·5%).

Ethylation of synthetic aspidinol (1 g.) with ethyl iodide (2 c.c.) and potassium carbonate (2 g.) in boiling acetone (25 c.c.) during 5 hours gave 2-hydroxy-4-methoxy-6-ethoxy-3-methyl-*n*-butyrophenone (IV), which separated from light petroleum (b. p. 60—80°) in elongated rectangular plates, m. p. 114°, identical in every way with an authentic specimen (Found : C, 66·4; H, 8·0%).

Natural Aspidinol.—Specimen (A) (see p. 820), which partly melted at 138°, was recrystallised from benzene and on heating became semi-solid at 140—141° and finally melted at 156°. Mixed with pure synthetic material, it behaved in the same manner. Specimen (B), m. p. 138—140°, had m. p. 142° after crystallisation from benzene and was identical in every way with a synthetic specimen. Specimen (C) had m. p. 132—135° and appeared to be mainly

aspidinol. The natural and the synthetic specimens had identical ferric chloride reactions—addition of 1 or 2 drops of 2·5% aqueous ferric chloride to an alcoholic solution of the ketone gave a dark green coloration which changed to brownish-violet on dilution with water.

Ethylation of (B) or (C) as in the case of the synthetic material gave rise to the ethyl ether, m. p. and mixed m. p. 114°; on cooling, the mixed melt solidified, and this solid again melted at the same temperature.

Acetylation of (A) gave an acetate which, once recrystallised, had m. p. 56°, but lack of material prevented further purification. Mixed with synthetic diacetate, it melted at 58—60°.

2-Hydroxy-4 : 6-dimethoxy-3-methyl-n-*butyrophenone*.—In the preparation of *C*-methylphloro-*n*-butyrophenone improved yields were obtained when the solution of the crude ketimine (from 6 g. of methylphloroglucinol and 5 g. of *n*-butyronitrile) in water (300 c.c.) was neutralised with aqueous ammonia (*d* 0·880) and heated on the steam-bath for 1·5 hours (compare Karrer, *Helv. Chim. Acta*, 1919, **2**, 466). Yield of hydrate, 5·9 g.

(1) Methylation of *C*-methylphloro-*n*-butyrophenone (2 g.) with methyl iodide (4 c.c.) and potassium carbonate (4 g.) in boiling acetone (30 c.c.) during 5 hours gave the *ketone*, which separated from ligroin or alcohol in colourless slender needles, m. p. 111—112° (Found: C, 65·3; H, 7·7. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6%). With alcoholic ferric chloride this compound gives a red-brown coloration. The *acetate* crystallised from ligroin in thick rectangular prisms, m. p. 61—62° (Found: C, 64·3; H, 7·4. $C_{15}H_{20}O_5$ requires C, 64·0; H, 7·1%).

(2) A mixture of phloro-*n*-butyrophenone (Robertson and co-workers, J., 1931, 1252) (8 g.), methyl iodide (20 c.c.), potassium carbonate (20 g.), and acetone (60 c.c.) was heated on the water-bath for 8 hours, and on crystallisation from alcohol the product formed slender needles, m. p. and mixed m. p. 111—112°; acetate, m. p. and mixed m. p. 61—62°.

5 : 7-Dimethoxy-2 : 8-dimethyl-3-ethylchromone.—Treatment of the foregoing ketone (2·5 g.) with acetic anhydride (25 c.c.) and sodium acetate (3 g.) at 200—210° for 16 hours gave the *chromone*, which separated from alcohol (charcoal) and then from light petroleum (b. p. 80—100°) in colourless rectangular plates, m. p. 171—172° (Found: C, 68·4; H, 6·8. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%). The pale yellow solution of the compound in concentrated sulphuric acid has a faint green fluorescence.

Methylation of C-*Methylphloro*-n-*butyrophenone with Diazomethane*.—A solution of the anhydrous ketone (3·5 g.) was treated at 0° with ethereal diazomethane (from 4·2 c.c. of nitrosomethylurethane), added in 6 portions, and next day the product was triturated with 10% sodium carbonate solution, washed, and dissolved in hot 50% alcohol. On cooling, the solution deposited 2-hydroxy-4 : 6-dimethoxy-3-methyl-*n*-butyrophenone in colourless needles, m. p. and mixed m. p. 111—112° after recrystallisation [Found: OMe, 25·6. Calc. for $C_{11}H_{12}O_2(OMe)_2$: OMe, 26·1%].

Addition of water to the alcoholic mother-liquor precipitated aspidinol, m. p. 130—135° after crystallisation from xylene. Repeated crystallisation from benzene, followed by sublimation in a high vacuum, finally gave this ketone in yellow plates, m. p. 142°, identical in every way with an authentic specimen [Found: C, 64·2; H, 7·3; OMe, 13·0. Calc. for $C_{11}H_{13}O_3(OMe)$: C, 64·3; H, 7·1; OMe, 13·8%].

The authors are indebted to the Chemical Society and to the Dixon Fund of London University for research grants.

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[*Received, April 8th*, 1933.]