763. *The Chemistry of Fungi. Part XLVI.*[1] *The Constitution of Ergoflavin*

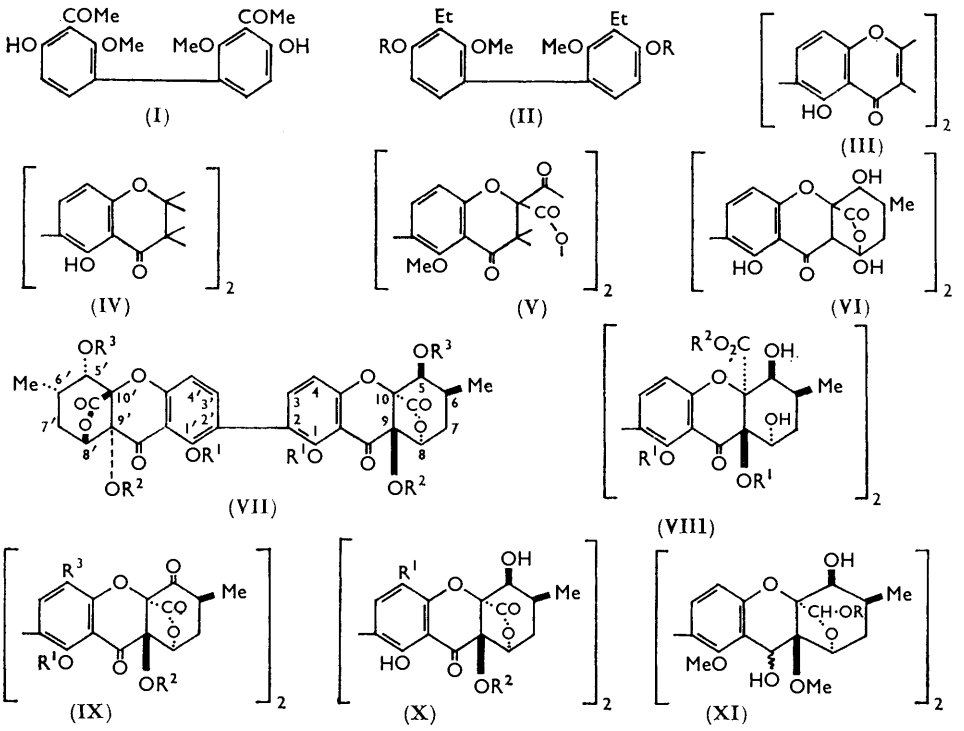
By J. W. ApSimon, J. A. Corran, N. G. Creasey, K. Y. Sim, and W. B. Whalley

The diphenyl derivative previously obtained [2] by alkali degradation of tetra-*O*-methylergoflavin has been defined as 3,3′-diacetyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl (I). The same derivative, together with oxalic acid is formed by the action of alkali upon tetra-*O*-methylergoflavinone (IX; R^1 = R^2 = Me, R^3 = H), an oxidation product of tetra-*O*-methylergoflavin (VII; R^1 = R^2 = Me, R^3 = H).

An extensive examination of the chemistry and spectral properties of ergoflavin and its numerous derivatives in conjunction with an *X*-ray analysis has enabled the constitution of ergoflavin to be defined as (VII; R^1 = R^2 = R^3 = H). The structures of the various degradation products follow.

Ozonolysis of ergoflavin disrupts one half of the symmetrical molecule to give hemiergoflavin-2-carboxylic acid (XXI; R^1 = H, R^2 = CO_2H) which can be decarboxylated to hemiergoflavin (XXI; R^1 = R^2 = H).

In a preliminary report [2] concerning the principal constituent, ergoflavin, isolated from the complex mixture of ergot pigments, we defined the major functional features of this molecule and proposed the most probable molecular formula as $C_{30}H_{26}O_{14}$. Continuing our comprehensive investigation of this and the associated metabolites we have confirmed



this formula and have defined the structure (but not all the stereochemical assignments) of ergoflavin as (VII; R^1 = R^2 = R^3 = H). In collaboration with Professor J. M.

[1] Part XLV, J. N. Chatterjea and W. B. Whalley, preceding Paper.

[2] G. Eglinton, F. E. King, G. Lloyd, J. W. Loder, J. R. Marshall, A. Robertson, and W. B. Whalley, *J.*, 1958, 1833.

Robertson, F.R.S., and his colleagues of the University of Glasgow, to whom we express our gratitude, this structure has been substantiated and the total absolute stereochemistry defined as in (VII; $R^1 = R^2 = R^3 = H$). Preliminary reports of these findings have been published.[3] Ergoflavin is thus the first representative of a series of closely related compounds which contain the bis(hexahydroxanthonyl) system.* The numbering of ergoflavin, as in (VII), is therefore based upon that of xanthone. We now report the chemical evidence for this constitution.

In our initial work [2] it was observed that degradation of tetra-*O*-methylergoflavin (VII; $R^1 = R^2 = Me$, $R^3 = H$) with baryta gave a product, $C_{16}H_{12}O_4(OMe)_2$, which was identified as a di-*O*-methyl ether of 3,3'-diacetyl-2,2',4,4'-tetrahydroxybiphenyl. The constitution of this moiety has now been defined as 3,3'-diacetyl-4,4'-dihydroxy-2,2'-dimethoxybiphenyl (I). Thus, reduction of (I) gave 3,3'-diethyl-4,4'-dihydroxy-2,2'-dimethoxybiphenyl (II; R = H) which was converted into 4,4'-diethoxy-3,3'-diethyl-2,2'-dimethoxybiphenyl (II; R = Et) identical with an authentic specimen.[5] The isolation of this degradation product supported our earlier conclusion [2] that the molecule of ergoflavin is symmetrical.

The formation of the biphenyl (I) is reminiscent of the production of *o*-hydroxyacetophenones from flavonoids by the action of bases. Since the infrared spectrum of ergoflavin and its derivatives indicated the presence of a chelated carbonyl group in a six-membered ring in conjugation with an aromatic system, it was reasonable to expand the partial formula for ergoflavin to (III) or (IV), *i.e.*, to a bis-chromone or a bis-chromanone type.

Because the biphenyl (I) contains only two of the four methoxyl groups of tetra-*O*-methylergoflavin (VII; $R^1 = R^2 = Me$, $R^3 = H$) it seemed probable that the second pair of methoxyls was remote from the aromatic nucleus. This is in agreement with the general properties of, *e.g.*, di-*O*-methylergoflavin (VII; $R^1 = R^3 = H$, $R^2 = Me$) which has an intense green ferric reaction and exhibits chelated carbonyl absorption at 1623 $cm.^{-1}$ in the infrared spectrum.[2] It further follows that the two methoxyl groups introduced into di-*O*-methylergoflavin in the course of its conversion into tetra-*O*-methylergoflavin are those which appear in the biphenyl (I). Clarification of this point was achieved as follows. Di-*O*-ethylergoflavin (VII; $R^1 = R^3 = H$, $R^2 = Et$) was methylated to yield the di-*O*-ethyldi-*O*-methylergoflavin (VII; $R^1 = Me$, $R^2 = Et$, $R^3 = H$) which was degraded directly, or preferably by way of the corresponding ergoflavinone (see later), to the biphenyl (I), thereby affording unequivocal evidence that the alkyl residues in the di-*O*-alkyl derivatives of ergoflavin are not attached to the biphenyl nucleus and that the methoxyl groups introduced secondarily are those which persist in the fragment (I). These methoxyls are therefore located at the 5-positions of the chromone (III) or chromanone (IV) systems. The presence of a bis-chromanone rather than of a bis-chromone system was inferred from the infrared spectra of ergoflavin and its derivatives (Table 1). These data reveal that the strong absorption band of the chelated *o*-hydroxycarbonyl system in the 1620—1650 $cm.^{-1}$ region of the spectra of ergoflavin and its di-*O*-alkyl derivatives is shifted to the 1690 $cm.^{-1}$ region when these compounds are methylated or acetylated. This behaviour is characteristic of simple *o*-hydroxyacetophenones,[6,7] *o*-hydroxybenzaldehydes,[6] and 5-hydroxyflavanones,[6] where, because of strong internal hydrogen

* Additional representatives are described in the following Paper,[4] whilst further examples are to be reported by Professor P. de Mayo (personal communication from Professor de Mayo).

[3] J. W. ApSimon, J. A. Corran, N. G. Creasey, K. Y. Sim, and W. B. Whalley, *Proc. Chem. Soc.*, 1963, 209; J. D. M. Asher, A. T. McPhail, J. M. Robertson, J. V. Silverston, and G. A. Sim, *ibid.*, p. 210.

[4] Part XLVII, J. W. ApSimon, J. A. Corran, N. G. Creasey, W. Marlow, W. B. Whalley, and (in part) K. Y. Sim, following Paper.

[5] J. W. ApSimon, N. G. Creasey, W. Marlow, K. Y. Sim, and W. B. Whalley, Part XLVIII, *J.*, 1965, 4156.

[6] K. Y. Sim and W. B. Whalley, unpublished results.

[7] W. Baker, A. C. M. Finch, W. D. Ollis, and K. W. Robinson, *J.*, 1963, 1477.

bonding, the carbonyl band occurs at lower frequencies, but upon either *O*-acetylation or *O*-alkylation the carbonyl band is shifted to higher (normal) frequencies. Conversely, *O*-acylation or *O*-alkylation of 5-hydroxy-chromones or -flavones produces the opposite effect,[6,7] *i.e.*, the carbonyl frequencies of the 5-acyloxy- and 5-alkoxy-chromones are shifted to frequencies lower than those exhibited by the parent hydroxy-compounds. Since the shift for the ergoflavin derivatives is always to higher frequencies, it was inferred that ergoflavin has the bis-chromanone nucleus (type IV) rather than the bis-chromone system (type III).

TABLE 1

Infrared data

	A	B	C	D
Ergoflavin	1800			1642
Di-*O*-methylergoflavin	1800			1623
Di-*O*-ethylergoflavin	1810			1630
Dibromo-di-*O*-methylergoflavin	1800			1634
Dinitroergoflavin *	1802			1656
Ergoflavinone (keto-form)	1815	1740		1642
Di-*O*-methylergoflavinone	1805	1750		1618
Tetra-*O*-acetyldi-*O*-methylergoflavin	1812	1786	1692	
		1757		
Tetra-*O*-acetyldi-*O*-methylergoflavinone	1815	1775	1690	
Tetra-*O*-methylergoflavin	1800		1690	
Di-*O*-acetyltetra-*O*-methylergoflavin	1805	1745	1692	
Di-*O*-*p*-iodobenzoyltetra-*O*-methylergoflavin	1808	1727	1690	
Tetra-*O*-methylergoflavinone	1808	1753	1698	
Di-*O*-acetyltetra-*O*-methylergoflavinone	1802	1770	1690	
Tetra-*O*-methyldinitroergoflavinone	1808	1750	1695	
Di-*O*-ethyldi-*O*-methylergoflavinone	1800	1745	1695	
Hexa-acetylergoflavinone	1815	1785	1698	

* Spectrum measured in chloroform. Band frequencies are given in wave numbers. A = γ-lactone, B = ester and aliphatic ketone, C = non-chelated aryl ketone, D = chelated aryl ketone

Fusion of ergoflavin with alkali gives (±)-methylsuccinic acid.[2] This fact, together with the n.m.r. spectrum of, *e.g.*, tetra-*O*-methylergoflavin which exhibits *inter alia* a broad complex signal centred at *ca.* τ 8·0 (7- and 7′-methylene protons) and a doublet at τ 8·81 (J = 6 c./sec., C-6 and C-6′, $>$CHMe, 6 protons), conclusively indicated the presence of the residue C·CH(Me)·CH_2·C in the metabolite.

Oxidation of tetra-*O*-methylergoflavin with the Jones reagent [8] gives tetra-*O*-methylergoflavinone (IX; $R^1 = R^2 = Me$, $R^3 = H$). This oxidation proceeds satisfactorily only if the reagent is freshly prepared. The n.m.r. spectrum of tetra-*O*-methylergoflavinone shows no aldehydic protons, and the signal associated with the $>$CHMe residues has shifted downfield to τ 8·71 (J = 6 c./sec.). It therefore follows (a) that the alcoholic hydroxyl groups must be secondary, and (b) that the new carbonyl groups are adjacent to the $>$CHMe residues. In contrast to tetra-*O*-methylergoflavin, alkali degradation of tetra-*O*-methylergoflavinone proceeds rapidly to furnish the biphenyl (I) in high yield together with *ca.* 1·25 molecular proportions of oxalic acid. In conjunction with the n.m.r. spectrum of, *e.g.*, tetra-*O*-methylergoflavin which has *inter alia* two pairs of doublets at τ 2·44 and 2·48 (J = 9 c./sec., two *ortho*-aromatic protons), this reaction clearly rules out the possibility that (I) might be an artifact. The presence of the residue (V) in tetra-*O*-methylergoflavinone was thus clearly indicated (cf. the analogous rotenonone).[9] The terminus of the γ-lactone bridge therefore had to be located on the remaining carbon atom. Hence, ergoflavin could be represented as (VI) or (VII; $R^1 = R^2 = R^3 = H$) (without stereochemical assignment).

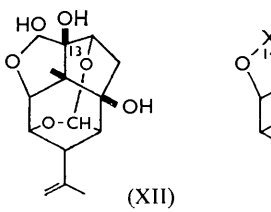
Unequivocal differentiation between these alternatives was not readily possible. Thus, although (VI), as a hemiacetal, would account for the acidity of the non-phenolic hydroxyl

[8] A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J.*, 1953, 2548.

[9] Cf. W. B. Whalley, in " Heterocyclic Compounds," ed. R. C. Elderfield, Wiley, New York, 1961, vol. 7, p. 169.

groups, this formulation is not in accord with, *e.g.*, the properties of ergoflavinic acid or its dimethyl ester.[2] On the other hand, formula (VII) does not offer any convincing *a priori* certainty for the acidity of the 9- and 9′-hydroxyl groups. At this stage an unequivocal definition became possible from the work of the Glasgow group who defined the structure and absolute stereochemistry of the di-*p*-iodobenzoate from tetra-*O*-methylergoflavin as (VII; $R^1 = R^2 = Me$, $R^3 = p$-iodobenzoyl), whence ergoflavin is represented by (VII; $R^1 = R^2 = R^3 = H$). It thus follows that the di-, tri-, and tetra-*O*-methyl ethers of ergoflavin are the 9,9′-di-*O*-methyl (VII; $R^1 = R^3 = H$, $R^2 = Me$), the 1,9,9′-tri-*O*-methyl, and the 1,1′,9,9′-tetra-*O*-methyl (VII; $R^1 = R^2 = Me$, $R^3 = H$) derivatives, respectively.

The structures of many other derivatives are obvious; *e.g.*, di-*O*-acetyl tetra-*O*-methylergoflavin is (VII; $R^1 = R^2 = Me$, $R^3 = Ac$), whilst ergoflavinic acid [2] and its dimethyl ester [2] are represented by (VIII; $R^1 = R^2 = H$) and (VIII; $R^1 = H$, $R^2 = Me$), respectively; tetra-*O*-methylergoflavinic acid is (VIII; $R^1 = Me$, $R^2 = H$) and the dimethyl ester is (VIII; $R^1 = R^2 = Me$). Tetra-*O*-methylergoflavinone and ergoflavinone are (IX; $R^1 = R^2 = Me$, $R^3 = H$) and (IX; $R^1 = R^2 = R^3 = H$), respectively. Since ergoflavin and its derivatives are devoid of protons *ortho* to the 1- and 1′-hydroxyl (or alkoxyl) substituents, nitration and bromination will result in *para*-substitution in each benzenoid nucleus. Thus, dinitroergoflavin [2] (X; $R^1 = NO_2$, $R^2 = H$) and dinitro-9,9′-di-*O*-methylergoflavin (X; $R^1 = NO_2$, $R^2 = Me$) (both exhibit negative Gibbs tests), diaminoergoflavin (X; $R^1 = NH_2$, $R^2 = H$), dibromo-9,9′-di-*O*-methylergoflavin (X; $R^1 = Br$, $R^2 = Me$), and dinitrotetra-*O*-methylergoflavinone (IX; $R^1 = R^2 = Me$, $R^3 = NO_2$) have the structures indicated.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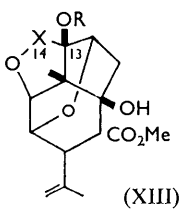
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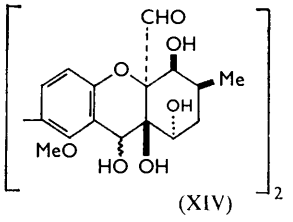
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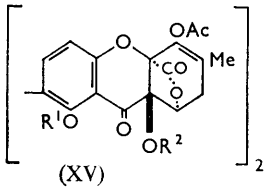
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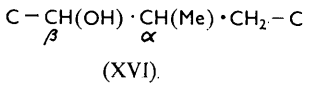
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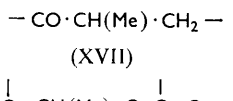
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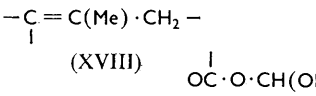
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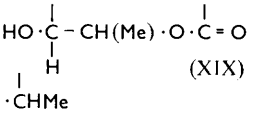
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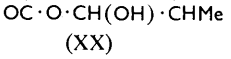
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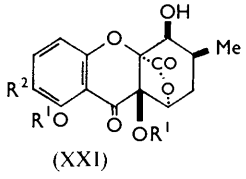
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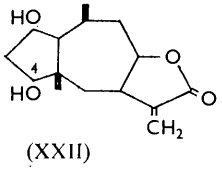
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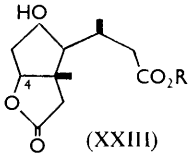
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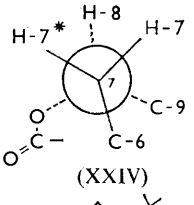
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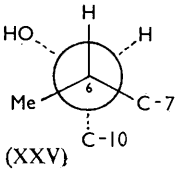
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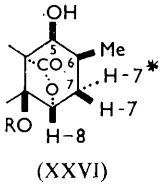
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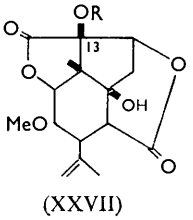
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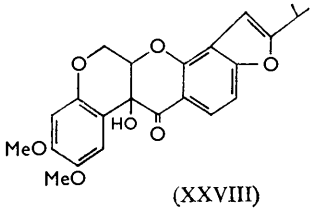

(XXVIII)

Tetra-*O*-methylergoflavol,[2] $C_{30}H_{30}O_{10}(OMe)_4$, the lithium aluminium hydride reduction product of tetra-*O*-methylergoflavin, is soluble in alkali and is converted by the action of dimethyl sulphate and alkali into hexa-*O*-methylergoflavol.[2] Since the infrared spectrum is devoid of carbonyl absorption, tetra-*O*-methylergoflavol is formulated as (XI; R = H) in which the acidity is attributed to the hemiacetal system [cf. the reduction, by the action of lithium aluminium hydride,[10] of the lactone, picrotoxinin, to the acidic hemiacetal (XII) and the acidity of the 14-hydroxyl group in the analogous methyl picrotoxolate (XIII; X = CH·OH, R = H)]. Demethylation of tetra-*O*-methylergoflavol with hydrochloric acid in acetic acid furnished a difficultly purifiable compound which appears to have the composition, $C_{30}H_{32}O_{12}(OMe)_2$. It is insoluble in sodium hydroxide solution, has carbonyl absorption at 1739 cm.$^{-1}$, and yields an amorphous dinitrophenylhydrazone. These properties suggest that it is the dialdehyde (XIV) derived by fission of the hemiacetal system in tetra-*O*-methylergoflavol.

Oxidation of ergoflavin with potassium permanganate yields (−)-methylsuccinic acid (*ca.* 1·5 moles). Since the absolute configuration of this acid has been defined,[11] the absolute stereochemistry of the metabolite at C-6 and C-6′ is as in (VII). This is in agreement with the *X*-ray analysis [3] and provides an unequivocal confirmation of the presently accepted absolute configuration of (−)-methylsuccinic acid.[11] Although there is no steric resistance to the introduction of a double bond between C-5 and C-6 [cf. the ease of formation of, *e.g.*, the enolic diacetate (XV; $R^1 = R^2 =$ Me) from tetra-*O*-methylergoflavinone (IX; $R^1 = R^2 =$ Me, $R^3 =$ H)], ergoflavin could not be dehydrated using acidic catalysts. It appears that elimination of water does not occur readily by an *E*1 mechanism, owing to the difficult energetics of carbonium ion formation at C-5 occasioned by the rigidity of the molecule. In addition the hexa-acetate (VII; $R^1 = R^2 = R^3 =$ Ac) is extremely stable under pyrolytic conditions. Because neither an *E*2 nor a pyrolytic elimination occurs readily, we deduce that the disposition of the substituents is such that the 5-hydroxyl is neither *trans* and anti-parallel nor *cis* and coplanar with a proton at C-7. Further the rigidity of the system must be sufficient (and models confirm this) to inhibit the formation of the requisite transition states. Additional consequences of this rigidity are apparent in the stability of the 5-hydroxyl group to boiling halogen acids [2] and the resistance of the α-ketol system in ergoflavin and the α-glycol residue in tetra-*O*-methylergoflavol to oxidation with periodic acid (cf. the analogous stability of certain α-glycols in which a *trans* disposition of hydroxyl groups is combined with structural rigidity [12]).

The high frequency (*ca.* 1800 cm.$^{-1}$) associated with the γ-lactone in ergoflavin and its derivatives (cf. Table 1) is to be ascribed, at least in part, to the strain imposed by the fused ring system (cf. Bellamy [13]). Several analogously constituted γ-lactones, *e.g.*, monascin [14,15] and picrotoxinin,[16] similarly exhibit abnormally high carbonyl frequencies. A contributory factor to the high frequency is the attachment of the electronegative ethereal oxygen atom to the α-carbon atom of the lactonic carbonyl residue. The effect of the electronegative substituent (devoid of stain effects) is apparent in, *e.g.*, the dimethyl ester of ergoflavinic acid [2] (VIII; $R^1 =$ H, $R^2 =$ Me) which has $\nu_{max.}$ 1740 cm.$^{-1}$. The relatively high frequencies ($\nu_{max.}$ 1740—1750 cm.$^{-1}$) of the 5-carbonyl residues in, *e.g.*, 9-9′-di-*O*-methylergoflavinone (IX; $R^1 = R^3 =$ H, $R^2 =$ Me) and in tetra-*O*-methylergoflavinone (IX; $R^3 =$ H, $R^1 = R^2 =$ Me) are similarly to be attributed to the combination

[10] J. S. E. Holker, K. U. Holker, A. McGookin, A. Robertson, K. Sargeant, and D. E. Hathway, *J.*, 1957, 3746.
[11] A. Fredga, *Arkiv. Kemi. Mineral Geol.*, 1947, **24***A*, 32.
[12] B. H. Alexander, R. J. Dimler, and C. L. Mehltretter, *J. Amer. Chem. Soc.*, 1951, **73**, 4658.
[13] L. J. Bellamy, " The Infra-red Spectra of Complex Molecules," Methuen, London, 1960, pp. 148, 187.
[14] B. C. Fielding, J. S. E. Holker, D. F. Jones, A. D. G. Powell, K. W. Richmond, A. Robertson, and W. B. Whalley, *J.*, 1961, 4579.
[15] Y. Inoye, K. Nakanishi, H. Nishikawa, M. Ohashi, A. Terahara, and S. Yamamura, *Tetrahedron*, 1962, **18**, 1195.
[16] R. M. Carman, R. G. Coombe, R. B. Johns, and A. D. Ward, *J.*, 1960, 1965.

of ring strain and substitution in the α-position by the ethereal oxygen atom of the central pyrone ring.

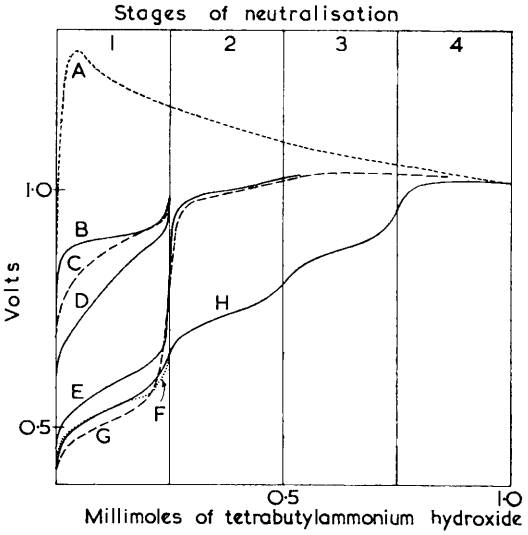
The infrared spectra of ergoflavin and its derivatives provide collateral support for the view that ether formation at C-9 and C-9′ precedes reaction at C-1 and C-1′. Thus, the spectrum of ergoflavin has hydroxyl absorption bands at 3500 and 3300 cm.$^{-1}$, whilst tetra-*O*-methylergoflavin and the dialkyl ethers of ergoflavin exhibit only one sharp hydroxyl peak at 3500 cm.$^{-1}$, which must be attributed to the secondary hydroxyl residues. This deduction is consistent with the observation (Table 1) that those derivatives which have free hydroxyl groups at C-1 and C-1′ show carbonyl absorption at frequencies which are 50—60 cm.$^{-1}$ lower (due to chelation) than those in which the 1- and 1′-hydroxyl residues are alkylated or acylated. For hydrogen bonding to be effective in an α-ketol, the five- and six-membered rings must be coplanar and even then the shifts are relatively small,[17] *e.g.*, isorotenolone A (XXVIII) has $\nu_{max.}$ 1676 cm.$^{-1}$. The corresponding *O*-methyl ether has $\nu_{max.}$ 1683 cm.$^{-1}$.

Ergoflavin is too sparingly soluble in the usual solvents for its n.m.r. spectrum to be determined satisfactorily, but valuable information was obtained from the spectra of its derivatives. Since ergoflavin is a symmetrical molecule the following comments refer to protons in one half of the molecule only. In addition to the pair of doublets at τ 2·48 and 3·02 (J = 8 c./sec., aromatic protons at C-3 and C-4), the n.m.r. spectrum of tetra-*O*-methylergoflavin (VII; $R^1 = R^2 = Me$, $R^3 = H$) exhibits a diffuse signal between τ 5·8 and 6·0 which we ascribe to the 5-proton. This assignment is confirmed by the absence of this signal in, *e.g.*, tetra-*O*-methylergoflavinone (IX; $R^3 = H$, $R^1 = R^2 = Me$) and by the large paramagnetic shift of this signal in the spectrum of 5,5′-di-*O*-acetyltetra-*O*-methylergoflavin (VII; $R^1 = R^2 = Me$, $R^3 = Ac$) to τ 4·84. Further evidence for the residue (XVII) in ergoflavinone and hence of (XVI) in ergoflavin is provided by the n.m.r. spectrum of 5,5′-di-*O*-acetyltetra-*O*-methylergoflavinone (XV; $R^1 = R^2 = Me$) where the vinyl acetoxyl residues are derived by enolisation of the 5-carbonyl residues. In this spectrum the >CHMe doublet is replaced by a singlet (τ 8·26, 3 protons) in accordance with the presence of the residue (XVIII). Similar effects are observed in the *C*-methyl signal of 9,9′-di-*O*-methylergoflavinone (IX; $R^1 = R^3 = H$, $R^2 = Me$) and of ergoflavinone (IX; $R^1 = R^2 = R^3 = H$) upon conversion into the tetra- and hexa-acetates (XV; $R^1 = Ac$, $R^2 = Me$) and (XV; $R^1 = R^2 = Ac$), respectively. These considerations clearly exclude the possibility of the α- and β-carbon atoms of the residue (XVI) corresponding to C-9 and C-10 of the ergoflavin system. The n.m.r. spectra of all derivatives of ergoflavin display a signal in the range τ 4·3—4·9 (1 proton) which is not exchangeable with deuterium oxide, and appears variously as a doublet or an ill-defined multiplet. This signal which is assigned to the proton of the system H–C–O–CO, *i.e.*, to the 8-proton, could not be due to either the α- or β-methine proton in (XVI) since this would be incompatible with other n.m.r. evidence, *e.g.*, the presence of the residues (XIX) or (XX) in tetra-*O*-methylergoflavin would be inconsistent with the persistence of this signal in tetra-*O*-methylergoflavinone and the diacetate (XV; $R^1 = R^2 = Me$). The presence of the residue (XIX) or (XX) is also chemically untenable since neither could give rise to methylsuccinic acid. The τ value of the 8-proton is in agreement with that of similarly situated protons (cf. *e.g.*, various gibberellic acid derivatives,[18] tenulin and its derivatives,[19] and avenaciolide [20]). The n.m.r. data are thus in agreement with the location of the non-phenolic hydroxyl groups at C-9 and C-9′.

Since the 7-methylene protons in ergoflavin and its derivatives are non-equivalent the 8-proton signal should consist of a pair of doublets (cf. pristimerin [21]). However, this

[17] L. Crombie and P. J. Godin, *Proc. Chem. Soc.*, 1960, 276.
[18] N. Sheppard, *J.*, 1960, 3041.
[19] W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *J. Amer. Chem. Soc.*, 1962, **84**, 3857.
[20] D. Brookes, B. K. Tidd, and W. B. Turner, *J.*, 1963, 5383.
[21] A. W. Johnson, P. F. Juby, T. J. King, and S. W. Tam, *J.*, 1963, 2884.

signal appears as only one doublet (J = 4 c./sec.) in tetra-*O*-methylergoflavin (and its 5,5′-di-*O*-acetyl derivative), di-*O*-methylhemiergoflavin (XXI; R^1 = Me, R^2 = H), and 2-methoxycarbonyldi-*O*-methylhemiergoflavin (XXI; R^1 = Me, R^2 = CO_2Me) (see later), thereby implying that one of the H-8/H-7 coupling constants is zero (cf. XXVI). It follows [22] that the H-7*/H-8 dihedral angle is *ca.* 90° (J = 0 c./sec.), and that the H-8/H-7 angle is *ca.* 35 or 155° (J = 4 c./sec.). Models indicate that ring c in these derivatives almost certainly has a distorted boat conformation with C-9 and C-6 at the stem and stern positions. In these circumstances the H-8/H-7* dihedral angle in (XXVI) is approximately 85° whilst the H-8/H-7 angle is about 35° [cf. the Newman projection (XXIV) along the C-7,C-8 axis]. Models also indicate that the dispositions of the substituents at C-6 and C-5 are as shown in the projection diagram (XXV), in agreement with the inferences from chemical evidence. The 8-proton signal in tetra-*O*-methylergoflavinone (IX; R^3 = H, R^1 = R^2 = Me), the corresponding enol acetate (XV; R^1 = R^2 = Me), 9,9′-di-*O*-methylergoflavinone (IX; R^1 = R^3 = H, R^2 = Me), and ergoflavinone hexa-acetate (XV; R^1 = R^2 = Ac) is



Potentiometric titration of ergoflavin with tetrabutylammonium hydroxide, in dimethylformamide

A, Solvent; B, 4,6-diethoxy-2-hydroxyacetophenone; C, phenol; D, 4-chloro-3-methylphenol; E, 2,2′,4,4′-tetrahydroxybiphenyl; F, benzoic acid; G, 9,9′-di-*O*-methylergoflavin; H, ergoflavin.

an ill-defined multiplet. It follows that the insertion of a trigonal atom at C-5 or of a double bond between C-5 and C-6 causes a considerable change in the conformation at C-7 and C-8. Models confirm this. A similar anomalous coupling behaviour is exhibited by certain derivatives of pulchellin (XXII) in which the 4-proton appears as a doublet, but in various transformation products, *e.g.*, (XXIII), the same proton furnishes a signal which consists of a pair of doublets.[23]

The unusual acidity of the tertiary alcoholic groups at C-9 and C-9′ in ergoflavin has been confirmed by potentiometric titration with tetrabutylammonium hydroxide in dimethylformamide (Figure). The acidity of at least one of these groups is evident from the titration curve (H) for ergoflavin which indicates a minimum of three acid groups per molecule. Furthermore, the second and third stages of the ergoflavin curve (H) may confidently be assigned to the tertiary alcoholic groups. Only two phenolic groups are available to account for the titration of 9,9′-di-*O*-methylergoflavin (G) and from a comparison of mid-point potentials, these two groups evidently correspond to the first and fourth stages of the ergoflavin curve. In the latter case failure to observe neutralisation

[22] H. Conroy, " Advances in Organic Chemistry, Methods and Results," ed. R. A. Raphael, Interscience, New York, 1960, Vol. II, p. 311.
[23] W. Herz, K. Ueda, and S. Inayama, *Tetrahedron*, 1963, **19**, 483.

of the second phenolic group in an obvious fourth stage of neutralisation is almost certainly due to alkali-metal-ion contamination [24] (see Experimental section) which reduced the voltage attainable at the alkaline end of the range. Nevertheless, it might also depend upon the structure of the reagent.[25]

A marked electrostatic interaction between the phenolic groups of 9,9′-di-*O*-methylergoflavin (and hence, by implication, between those of ergoflavin) is indicated by the large differences between the mid-point potentials of the two stages of the 9,9′-di-*O*-methylergoflavin neutralisation, one group being unusually strong and the other unusually weak [cf. curve (C) for phenol and curve (F) for benzoic acid in the Figure]. The curve (E) for 2,2′,4,4′-tetrahydroxybiphenyl similarly shows evidence of an equally large interaction between two of the four phenolic groupings, presumably those in the 2,2′-positions, which also possess strengths similar to those of 9,9′-di-*O*-methylergoflavin.

Although the orders of acid strengths observed in different solvents do not necessarily correspond,[26] the curves (C, D, and F) of the Figure show an order for dimethylformamide which is very similar to that for water. We may therefore infer that in water the tertiary alcoholic groups of ergoflavin posses the acidity of a typical phenol Furthermore, it is also apparent that if one or both of the lactone rings had been opened during titration by traces of water in the dimethylformamide the second stage of the ergoflavin neutralisation would have indicated an appreciably stronger acid group than that observed.

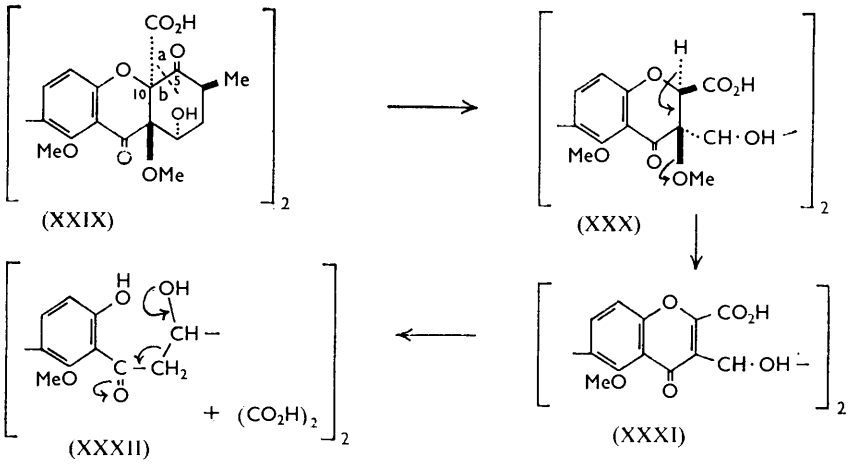
The acidity of the 9- and 9′-hydroxyl groups in ergoflavin constitutes one of the novel features of the metabolite, and is to be attributed, at least in part, to the conformation of ring c causing serious steric interaction between this hydroxyl group and the 6-methyl residue. The α-carbonyl function will provide supplementary activation. Both factors would tend to reduce the enthalpy of dissociation, and the accompanying entropy change might then assume a dominant role. It may be noted that hydroxyl groups adjacent to " acidifying " groups such as ester residues are sufficiently acidic to be methylated by diazomethane,[27] whilst in natural products the acidity of methyl picrotoxate (XIII; X = >C=O, R = H) (which is soluble in cold sodium hydroxide solution without opening of the lactone ring), has been attributed to the 13-hydroxyl function.[28] This hydroxyl can be methylated [28] with diazomethane or by dimethyl sulphate and alkali, to yield (XIII; X = >C=O, R = Me). The 13-hydroxyl group of apopicrotoxinin dilactone (XXVII; R = H) shows analogous acidity and can similarly be methylated [16] to yield (XXVII; R = Me). Since the α-ketol rotenolone (XXVIII) is apparently insoluble in alkali,[29] the effect of steric compression in ergoflavin must contribute significantly to the high degree of acidity of the 9-hydroxyl. In ergoflavinone (IX; $R^1 = R^2 = R^3 = H$) this steric compression is diminished but the great sensitivity of this compound to alkali made it impossible to assess the acidity of the 9-hydroxyl group by non-aqueous titration.

The formation of an *o*-hydroxyacetophenone (I) by the alkaline hydrolysis of, *e.g.*, tetra-*O*-methylergoflavinone (IX; $R^3 = H$, $R^1 = R^2 = Me$), rather than the corresponding *o*-hydroxy-ω-methoxyacetophenone, is to be ascribed to the stereochemistry of the parent molecule. The first stage in the degradation is probably opening of the lactone ring to yield (XXIX). Since oxalic acid is formed in high yield, hydrolytic cleavage must then occur substantially between C-5 and C-10 [*i.e.*, across the broken line ab in (XXIX)], to be followed by the elimination of methanol as in (XXX).

It follows that (a) inversion of configuration probably occurs at C-10 during the intial cleavage (this would accord with the bulky carboxyl group assuming the more stable

[24] G. A. Harlow, *Analyt. Chem.*, 1962, **34**, 148.
[25] G. A. Harlow, *Analyt. Chem.*, 1962, **34**, 1482.
[26] H. C. Brown, D. H. McDaniel, and O. Häfliger, " Determination of Organic Structures by Physical Methods," ed. E. A. Braude and F. C. Nachod, Academic Press, New York, 1955, p. 617.
[27] B. Eistert, " Newer Methods of Preparative Organic Chemistry," Interscience, New York, 1947, p. 520.
[28] P. I. Burkhill, J. S. E. Holker, A. Robertson, and J. H. Taylor, *J.*, 1957, 4945.
[29] F. B. La Forge and H. L. Haller, *J. Amer. Chem. Soc.*, 1934, **56**, 1620.

quasi-equatorial position), and (b) the pendant remnant of ring c remains quasi-equatorial, thereby maintaining the axial conformation of the 9-methoxyl residue. The final stages in the formation of (I) are indicated by the sequence (XXX) ⟶ (XXXII). The degradation [2] of, *e.g.*, tetra-*O*-methylergoflavin to the *o*-hydroxyacetophenone (I) most



probably depends upon the prior aerial oxidation of the secondary hydroxyl group, a view which rationalises the low yield of (I) obtained by this route.

Ozonolysis of ergoflavin destroys half of the molecule with the formation of hemiergoflavin-2-carboxylic acid (XXI; $R^1 = H$, $R^2 = CO_2H$). The methyl ester (XXI; $R^1 = H$, $R^2 = CO_2Me$) has $\nu_{max.}$ 1720 (aryl ester) cm.$^{-1}$. Decarboxylation of (XXI; $R^1 = H$, $R^2 = CO_2H$) gives hemiergoflavin, (XXI; $R^1 = R^2 = H$). Attempts at oxidative coupling of this material have not yet succeeded. Methylation of hemiergoflavin gives di-*O*-methylhemiergoflavin (XXI; $R^1 = Me$, $R^2 = H$) the infrared and n.m.r. spectra of which are very similar to those of tetra-*O*-methylergoflavin. Oxidation of di-*O*-methylhemiergoflavin gives di-*O*-methylhemiergoflavinone.

The intensities of the ultraviolet absorption maxima in biflavonyls in which the flavonoid chromophores are insulated from each other are approximately twice those in

TABLE 2

Compounds	$\lambda_{max.}$ (mμ) and $\varepsilon_{max.}$		
Ergoflavin	242(21,400)	278(21,200)	384(7900)
Hemiergoflavin		282(6000)	370(2400)
2-Hydroxy-6-methoxyacetophenone		272(10,700)	335(3300)
3,3′-Diacetyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl	248(30,900)		346(3980)
5-Hydroxy-2-methylchromone *	252(12,300)	325(4270)	226(20,400)
8,8′-Di-(5-hydroxy-2-methylchromone) *	242(35,000)	335(9550)	
6,6′-Di-(5-hydroxy-2-methylchromone) *	243(34,700)	335(8700)	

* B. Franck and G. Baumann, *Chem. Ber.*, 1963, **96**, 3209.

the corresponding flavones.[7] The ultraviolet data for ergoflavin, hemiergoflavin, and some model compounds are listed in Table 2, from which it is apparent that the intensities of absorption for ergoflavin are approximately three times those for the corresponding bands of hemiergoflavin. The model compounds show similar behaviour. These data are thus in accord with the structure of ergoflavin.

On general grounds it would be expected that the two halves of the ergoflavin molecule would be non-planar. The *X*-ray analysis [3] confirms this deduction which is available independently from n.m.r. spectra. Thus, the methoxyl signals for 2-methoxycarbonyldi-*O*-methylhemiergoflavin (XXI; $R^1 = Me$, $R^2 = CO_2Me$) and di-*O*-methylhemiergoflavin (XXI; $R^1 = Me$, $R^2 = H$) occur in the regions τ 6·1 and 6·6, whereas the corresponding

signals for tetra-*O*-methylergoflavin (VII; $R^1 = R^2 = Me$, $R^3 = H$) and its derivatives are located in the regions τ 6·45 and 6·6. In both series it is apparent that the signal at τ 6·6 is that of the 9-methoxyl group. The diamagnetic shift (0·36 p.p.m.) of the 1-methoxyl signal of tetra-*O*-methylergoflavin relative to that of 9,9′-di-*O*-methylhemiergoflavin is indicative of a large dihedral angle between the two benzene rings of the biphenyl nucleus, which results in a mutual shielding of the 1-methoxyl residue in one half of the molecule by the aromatic ring of the other half. This upfield shift of signals associated with methoxyl residues in " hindered " positions in biphenyl systems has been observed with other natural products, *e.g.*, podototarin derivatives,[30] macrophyllic acid,[31] and the methyl ethers of bulbocapnine and glaucine.[32] Further evidence for a large dihedral angle between the aromatic rings in ergoflavin and its derivatives is provided by the n.m.r. spectra of various acetyl derivatives. Thus, although the contrary might be anticipated, the 1-acetoxyl signal (τ 7·86) of 1,1′,5,5′-tetra-*O*-acetyl-9,9′-dimethylergoflavinone (XV; $R^1 = Ac$, $R^2 = Me$) is located at a higher field than the 5-vinyl acetoxyl signal (τ 7·67). This assignment of the latter signal is supported by the locations of the same signal at τ 7·64 for 5,5′-di-*O*-acetyl-1,1′,9,9′-tetra-*O*-methylergoflavinone (XV; $R^1 = R^2 = Me$) and 1,1′,5,5′,9,9′-hexa-acetylergoflavinone (XV; $R^1 = R^2 = Ac$), respectively. The diamagnetic shift of the 1-acetoxyl group is to be attributed to the shielding effect of the adjacent aromatic ring, from which it follows that the dihedral angle between the nuclei must be large.

It may be noted that the methoxyl signal of the tetra-acetate of di-*O*-methylergoflavinone appears at τ 6·63, in agreement with the evidence previously adduced for the structures of the di-*O*-alkyl ethers of ergoflavin.

A possible biosynthetic route to ergoflavin and its congeners is discussed in the following Paper.[4]

Experimental

Infrared spectra were determined in Nujol, using Infracord 137 and 237 spectrometers. Ultraviolet spectra were recorded for solutions in alcohol using a Perkin-Elmer U.V. 137 spectrometer. The n.m.r. spectra were determined in deuteriochloroform solution by Miss J. Lovenack using an A. 60 Varian spectrometer.

Light petroleum refers to the fraction of b. p. 60—80°. Alkoxyl analyses upon mixed methyl ethyl ethers are recorded as the total, equivalent, methoxyl content.

3,3-*Diethyl*-4,4′-*hydroxy*-2,2′-*dimethoxybiphenyl*.—3,3′-Diacetyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl (0·4 g.) was reduced by the method described previously.[2] The crude product was purified from benzene by chromatography on silica followed by elution with benzene–chloroform (9 : 1), to give 3,3′-*diethyl*-4,4′-*dihydroxy*-2,2′-*dimethoxybiphenyl*, needles (0·24 g.), m. p. 159° (from benzene–light petroleum) [Found: C, 71·3; H, 7·3; OMe, 20·4. $C_{16}H_{16}O_2(OMe)_2$ requires C, 71·5; H, 7·3; OMe, 20·5%]. Methylation of this biphenyl (75 mg.) by the dimethyl sulphate–acetone–potassium carbonate process gave 3,3′-diethyl-2,2′,4,4′-tetramethoxybiphenyl (60 mg.) which formed prisms, m. p. 108° (from light petroleum), identical with a previously prepared specimen.[2]

Ethylation of 3,3′-diethyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl (117 mg.), in a similar manner, gave 4,4′-*diethoxy*-3,3′-*diethyl*-2,2′-*dimethoxybiphenyl* (80 mg.), m. p. 136° [from light petroleum in prisms, or methanol in plates (cf. ref. 5] (Found: C, 73·8; H, 8·6. $C_{22}H_{30}O_4$ requires C, 73·7; H, 8·4%).

1,1′-*Di*-O-*ethyl*-9,9′-*di*-O-*methylergoflavin* (VII; $R^1 = Et$, $R^2 = Me$, $R^3 = H$).—A solution of 9,9′-di-*O*-methylergoflavin, $[\alpha]_D^{18} +15·3°$ (*c* 0·73 in $CHCl_3$), $\lambda_{max.}$ 245sh, 283, and 398 (log ε 4·19, 4·23, and 3·69) (1·9 g.), in acetone (250 ml.) containing diethyl sulphate (2·5 ml.) and potassium carbonate (4 g.), was refluxed for 15 hr. Extensive purification of the product by a combination of crystallisation and chromatography gave 9,9′-di-*O*-methylergoflavin (0·2 g.) and 1,1′-*di*-O-*ethyl*-9,9′-*di*-O-*methylergoflavin* (VII; $R^1 = Et$, $R^2 = Me$, $R^3 = H$) (0·2 g.) which formed pale

[30] R. C. Cambie, W. R. J. Simpson, and L. D. Colebrook, *Tetrahedron*, 1963, **19**, 209.
[31] S. M. Bocks, R. C. Cambie, and T. Takahasi, *Tetrahedron*, 1963, **19**, 1109.
[32] S. Goodwin, J. N. Schoolery, and L. F. Johnson, *Proc. Chem. Soc.*, 1958, 306.

yellow needles, m. p. 280° (decomp.) (from acetone–methanol), having a negative ferric reaction in alcohol [Found: C, 62·1; H, 5·5; OMe, 17·5. $C_{30}H_{22}O_{10}(OMe)_2(OEt)_2$ requires C, 62·3; H, 5·5; OMe, 17·9%]. Various modifications of this process did not increase the yield.

Ethylation of Ergoflavin.—(*a*) A solution of ergoflavin (1 g.) in acetone (50 ml.) containing ethyl iodide (1 ml.) and potassium carbonate (2 g.) was refluxed for 24 hr., additional ethyl iodide (1 ml.) was added, and heating continued until a sample had no ferric reaction (21 hr.). Chromatography of the product on alumina from benzene gave *1,1′,9,9′-tetraethylergoflavin* (0·1 g.) which formed pale yellow prisms, m. p. 288° (decomp.) (from acetone–methanol) [Found: C, 62·8; H, 6·0; OEt, 24·4. $C_{30}H_{22}O_{10}(OEt)_4$ requires C, 63·2; H, 5·9; OEt, 24·9%].

(*b*) A solution of ergoflavin (5 g.) in acetone (100 ml.) containing diethyl sulphate (5 ml.) and potassium carbonate (10 g.) was refluxed for 3 hr. During this period a yellow deposit formed in the mixture. The solution was filtered and the solid leached with 2N-hydrochloric acid; the insoluble residue thus obtained was combined with that remaining on distillation of the acetone from the filtrate; these materials were allowed to stand for 12 hr. with water (100 ml.) containing a few drops of concentrated hydrochloric acid.

The resulting amorphous yellow powder was dissolved in hot dioxan (100 ml.) and treated with hot water (100 ml.). On cooling, 9,9′-di-*O*-ethylergoflavin (2·8 g.) separated in yellow needles, m. p. 260° (decomp.), exhibiting an intense green ferric reaction in alcohol. This ether was recrystallised by dissolving in a large volume of acetone, evaporating the solution to a small bulk, and adding warm methanol, when *9,9′-di-O-ethylergoflavin* separated in stout yellow needles, m. p. 260° (decomp.) [Found: C, 60·6; H, 5·7; OEt, 14·0. $C_{30}H_{24}O_{12}(OEt)_2$ requires C, 61·3; H, 5·1; OEt, 13·5%]. Methylation of this di-*O*-ethyl ether (4 g.) by the dimethyl sulphate–potassium carbonate–acetone method was complete in 3 hr. Purification from methanol gave *1,1′-di-O-methyl-9,9′-di-O-ethylergoflavin* (VII; R^1 = Me, R^2 = Et, R^3 = H) in prisms (2·3 g.), m. p. 180° (decomp.). Crystallisation from ethanol gave rods, m. p. 160° (decomp.). After drying at 140°/0·2 mm. for 5 hr. both specimens had m. p. 280° (decomp.) [Found: C, 61·7; H, 5·6; OMe, 17·5. $C_{30}H_{22}O_{10}(OEt)_2$ requires C, 62·3; H, 5·5; OMe, 17·9%].

The previous mixed ether (2·3 g.) was heated under reflux for 5 hr. with a solution of barium hydroxide octahydrate (30 g.) in water (60 ml.). After isolation in the usual manner the phenolic fraction was purified by chromatography from benzene on silica followed by crystallisation from light petroleum, to yield 3,3′-diacetyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl (20 mg.), m. p. 168°, identical (m. p., mixed m. p., infrared) with an authentic specimen.

Oxidation of Ergoflavin with Potassium Permanganate.—A solution of potassium permangate (20 g.) in water (40 ml.) was added during 6 hr. to a cooled stirred solution of ergoflavin (4 g.) in acetone (200 ml.), and next day the solution was clarified with sulphur dioxide and the acetone removed under reduced pressure. Exhaustive extraction of the aqueous solution with ether gave a semi-solid residue (2 g.) which was chromatographed on silica from benzene. Elution with chloroform–acetone (9 : 1) furnished (−)-methylsuccinic acid (0·25 g.), needles, m. p. 111° (from chloroform–light petroleum), identical with an authentic specimen, $[\alpha]_D^{20}$ −11·78° (*c* 0·89 in acetone), $[\alpha]_D^{20}$ −7·95° (*c* 1·02 in water) (Found: C, 45·7; H, 6·1. Calc. for $C_5H_8O_4$: C, 45·5; H, 5·1%).

Chromic Acid Oxidation of Tetra-O-methylergoflavin.—(*a*) Freshly prepared Jones reagent [8] (8 ml.) was added dropwise to a stirred solution of tetra-*O*-methylergoflavin (2 g.) in acetone (100 ml.). Next day the excess oxidising agent was decomposed by methanol and the product isolated in the usual manner to yield *tetra-O-methylergoflavinone* (1·8 g.), needles, m. p. 290° (decomp.) (from acetone–methanol of chloroform–light petroleum), $[\alpha]_D^{20}$ +200° (*c* 1·30 in acetone) [Found: C, 61·4; H, 4·5; OMe, 18·4. $C_{30}H_{18}O_{10}(OMe)_4$ requires C, 61·5; H, 4·6; OMe, 18·7%]. The compound is devoid of a ferric reaction in alcohol and gives an immediate non-crystallisable precipitate with Brady's reagent. Prepared by the pyridine–acetic anhydride method, the *di-O-acetyl derivative* formed prisms, m. p. 303—305° (decomp.) [Found: C, 60·8; H, 4·8. $C_{34}H_{22}O_{12}(OMe)_4$ requires C, 61·1; H, 4·6%].

(*b*) A solution of chromic oxide (0·5 g.) in acetic acid (50 ml.) was added to tetra-*O*-methylergoflavin (500 ml.) dissolved in acetic acid (30 ml.). Next day, excess of oxidising agent was destroyed by methanol and the green solution was evaporated to a small volume under reduced pressure. Addition of water to this residue gave a colourless precipitate which, after extensive purification as in (*a*), gave tetra-*O*-methylergoflavinone (50 mg.), identical with the previous preparation. A solution of tetra-*O*-methylergoflavinone (0·5 g.) in fuming nitric acid (15 ml.)

was kept at 20° for ½ hr., heated on a steam-bath for 5 min., and added to ice (50 g.). Purification of the precipitate from acetone–methanol gave *tetra*-O-*methyl*-4,4′-*dinitroergoflavinone* in needles (0·3 g.), m. p. 287° (decomp.) [Found: C, 54·1; H, 3·9; N, 3·9. $C_{34}H_{28}O_{14}(NO_2)_2(OMe)_4$ requires C, 54·3; H, 3·7; N, 3·7%].

Alkali Degradation of Tetra-O-*methylergoflavinone*.—Tetra-*O*-methylergoflavinone (2 g.) rapidly dissolved in warm 10% sodium hydroxide solution (80 ml.). The solution was then refluxed for 2 hr. in a stream of nitrogen. On isolation in the usual manner the phenolic fraction gave a semi-crystalline solid (0·64 g.) which was purified from aqueous methanol to give 3,3′-diacetyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl (0·4 g.), m. p. 168°, identical with an authentic specimen [Found: C, 65·7; H, 5·5; OMe, 18·1. Calc. for $C_{16}H_{12}O_4(OMe)_2$: C, 65·4; H, 5·5; OMe, 18·8%].

The acidic fraction was purified from chloroform to yield oxalic acid (0·25 g.), which had the requisite m. p., mixed m. p., and infrared spectrum, and was characterised by conversion into the *S*-benzylisothiouronium salt [Found: C, 51·2; H, 5·3; N, 13·1. Calc. for $(C_8H_{11}N_2S)_2(C_2O_4)$: C, 51·2; H, 5·3; N, 13·3%]. The oxalic acid formed needles of the dihydrate, m. p. 99° (from water) (Found: C, 20·0; H, 4·7; OMe, 0. Calc. for $C_2H_2O_4,2H_2O$: C, 19·1; H, 4·8%).

9,9′-*Di*-O-*methylergoflavinone* (IX; $R^1 = R^3 = H$, $R^2 = Me$).—(*a*) A solution of 1,1′,9,9′-tetramethylergoflavinone (1 g.) in a mixture prepared from acetic anhydride (12 ml.) and hydriodic acid (*d* 1·7; 12 ml.), was warmed on a steam-bath for 45 min. Yellow crystals separated from the initially clear solution. The product was purified from ethyl acetate to yield 9,9′-*di*-O-*methylergoflavinone* (0·65 g.), yellow needles, m. p. 330° (decomp.), having a green ferric reaction in alcohol, $[\alpha]_D^{20}$ +330° (*c* 0·881 in $CHCl_3$), $\lambda_{max.}$ 248, 284, and 398 mμ (log ε 4·39, 4·34, and 3·90) [Found: C, 60·4; H, 4·3; OMe, 10·4. $C_{30}H_{20}O_{12}(OMe)_2$ requires C, 60·6; H, 4·1; OMe, 9·8%].

Prepared by the pyridine–acetic anhydride method, the *tetra*-O-*acetyl derivative* formed prisms, m. p. 279—282° (from acetone–methanol) (Found: C, 60·1; H, 4·4 $C_{40}H_{34}O_{18}$ requires C, 59·8; H, 4·2%).

Remethylation of 9,9′-dimethylergoflavinone by various methods gave only a low yield of 1,1′,9,9′-tetramethylergoflavinone, together with much non-crystallisable material.

When a solution of di-*O*-methylergoflavinone (0·6 g.) in 50% aqueous potassium hydroxide (5 ml.) was refluxed for ½ hr. and the phenolic fraction isolated in the usual manner, 3,3′-*diacetyl*-2,2′,4,4′-*tetrahydroxybiphenyl* (0·03 g.) was obtained as needles, m. p. 249—250°, identical (m. p., mixed m. p., infrared) with a synthetic specimen [5] (Found: C, 63·4; H, 4·9. $C_{16}H_{14}O_6$ requires C, 63·6; H, 4·7%).

(*b*) Oxidation of the 9,9′-dimethylergoflavin (0·5 g.) in acetone (200 ml.) by the Jones reagent [8] (2·5 ml.) during 24 hr. gave 9,9′-dimethylergoflavinone (0·17 g.) in yellow needles, identical with the specimen from (*a*) (m. p., mixed m. p., infrared).

5,5′-*Di*-O-p-*iodobenzoyltetra*-O-*methylergoflavin*.—A mixture of tetra-*O*-methylergoflavin (5 g.), *p*-iodobenzoyl chloride (1·5 g.), and pyridine (10 ml.) was warmed on a steam-bath for 3 hr. Next day the crystalline solid (*p*-iodobenzoic anhydride, m. p. 240°) was collected and the filtrate diluted with water (100 ml.). The precipitate was collected, tritrated with 2N-sodium hydrogen carbonate, washed, dried, and purified from benzene–light petroleum, to give the *product* as needles (0·6 g.), m. p. 207—211°. This ester also formed prisms, m. p. 305—308° (from acetone–methanol). The dimorphic forms were interconvertible by recrystallisation and seeding with the requisite crystals, $[\alpha]_D^{20}$ −28·4° (*c* 1·90 in acetone) [Found: (for needles) C, 50·8; H, 3·5; I, 22·5; OMe, 11·0. $C_{44}H_{28}I_2O_{14}(OMe)_4$ requires C, 50·0; H, 3·5; I, 22·0; OMe, 10·8%].

1,1′,5,5′-*Tetra*-O-*acetyl*-9,9′-*di*-O-*methylergoflavin* (VII; $R^1 = R^3 = Ac$, $R^2 = Me$).—Prepared by the pyridine–acetic anhydride method from di-*O*-methylergoflavin (0·5 g.), the *product* formed prisms (0·25 g.), m. p. 290° (decomp.) (from acetone–methanol) (Found: C, 60·0; H, 5·1. $C_{40}H_{38}O_{18}$ requires C, 59·6; H, 4·7%).

A solution of bromine (0·8 g.) in acetic acid (10 ml.) was added to a stirred solution of 9,9′-di-*O*-methylergoflavin (0·5 g.) in acetic acid (100 ml.). Purification of the precipitate from acetone–methanol gave 4,4′-*dibromo*-9,9′-*di*-O-*methylergoflavin* (0·26 g.) in yellow needles, m. p. 330° [Found: C, 48·6; H, 3·6; OMe, 8·1; Br, 20·5. $C_{30}H_{22}O_{12}(OMe)_2$ requires C, 48·3; H, 3·5; OMe, 7·8; Br, 20·1%].

Ergoflavinone.—Oxidation of a solution of ergoflavin (1·5 g.) in acetone (50 ml.) with freshly prepared Jones reagent [8] (1·8 ml.) during 5 hr., followed by isolation in the usual manner, gave

ergoflavinone, which separated from methanol in yellow needles (0·1 g.), m. p. 325—330° (decomp.), $[\alpha]_D^{20}$ +330° (*c* 0·56 in acetone) (Found: C, 58·9; H, 4·4. $C_{30}H_{22}O_{14}$ requires C, 59·4; H, 3·7%). The product had an intense green ferric reaction in alcohol and gave an immediate amorphous precipitate with Brady's reagent.

The *hexa-acetate* separated from acetone–methanol in needles, m. p. 208° (decomp.) [Found: C, 57·5; H, 4·4. $C_{30}H_{16}O_6(OAc)_6$ requires C, 58·6; H, 4·0%].

9,9′-*Di*-O-*ethyl*-1,1′-*di*-O-*methylergoflavinone* (IX; R^1 = Me, R^2 = Et, R^3 = H).—Oxidation of 9,9′-di-*O*-ethyl-1,1′-di-*O*-methylergoflavin (0·4 g.) in acetone (30 ml.) with the Jones reagent [8] (2 ml.) gave 9,9′-*di*-O-*ethyl*-1,1′-*di*-O-*methylergoflavinone* in plates (0·3 g.), m. p. 271° (decomp.) (from acetone–methanol) [Found: C, 63·2; H, 4·8; OMe, 9·0; OEt, 18·0. $C_{30}H_{18}O_{10}(OMe)_2(OEt)_2$ requires C, 62·6; H, 4·9; OMe, 8·9; OEt, 17·9%]. Decomposition of this mixed ether (1 g.) on a steam-bath during 2 hr. with 1% sodium hydroxide solution (40 ml.) gave 3,3′-diacetyl-4,4′-dihydroxy-2,2′-dimethoxybiphenyl (0·15 g.) and oxalic acid (0·17 g.) identical with authentic specimens.

Non-aqueous Potentiometric Titrations.—Samples were dissolved in dimethylformamide (15 ml.) (previously dried over barium oxide and distilled) and titrated with 0·1N-tetrabutylammonium hydroxide (B.D.H. reagent) in benzene–methanol which was stored in a totally enclosed automatic burette–reservoir system and frequently standardised against AnalaR benzoic acid.

A Pye lithium glass electrode was used as indicator together with a silver–silver chloride electrode in 0·1M-aqueous tetraethylammonium chloride as reference. The construction and handling of this cell took account of the well established fact [24] that, in non-aqueous solution, the glass electrode shows extreme sensitivity to traces of sodium and potassium ions in the " parts per million " range. The electrodes were stored in distilled water and dried with paper tissue immediately prior to use. A sleeve-type liquid junction proved essential on account of the tendency for fibre junctions to accumulate (from the reagents) quantities of alkali-metal ions, which poisoned subsequent titrations. Maximum sensitivity of the glass electrode to alkali-metal ions was exhibited in the presence of excess of titrant. Under these conditions, whereas the addition to 0·4% v/v water reduced the cell e.m.f. by only 0·01 v, the subsequent introduction of 0·4% v/v 0·1N-aqueous potassium chloride reduced it by as much as 0·2 v.

The tetraethylammonium chloride did not significantly affect the glass electrode potential, neither did the absorption of atmospheric carbon dioxide constitute an appreciable hazard. Cell e.m.f. values were read from the expanded millivolt scale of a Pye Dynacap pH meter of adequately high input resistance, the scale agreeing with that of a Pye Precision Potentiometer to within 0·0005 v. The electrodes, when immersed in 0·1M-benzoic acid (AnalaR) in dimethylformamide, gave a mean e.m.f. of 0·383 v. Readings were stable, duplicate titrations of ergoflavin and 9,9′-di-*O*-methylergoflavin being consistent to within 0·001 v over the entire curve, apart from the terminal sections, where differences were of the order of 0·010 v.

Numerical information on mid-point potentials is omitted on account of alkali-metal-ion contamination, which distorts the titration curve through a possibly non-linear transformation of its ordinate scale. Nevertheless, the conclusions are adequately justified by the data presented in the Figure. In the present work, alkali-metal ions came mainly from the titrant, and in the absence of sample led to the highly distorted blank curve (A) of the Figure. From the close agreement between the voltage achieved in the terminal sections of the curves and the corresponding portions of the blank titration curve, the contributions of the samples to the total contamination were evidently rather less than that of the titrant. Differences between sample contributions were, however, detectable. The solvent contribution was probably small in view of the similar blank titration curves obtained from three different batches of dimethylformamide.

Conclusions drawn from the titration curves of the Figure depend upon large differences that are inexplicable in terms of alkali-metal-ion contamination. Furthermore, the use of approximately equal molarities in the titration of ergoflavin and 9,9′-di-*O*-methylergoflavin largely cancelled the effect of curve distortion upon the final comparison.

The Ozonolysis of Ergoflavin.—Ozonised oxygen was passed through a solution of ergoflavin (2 g.) in ethyl acetate (250 ml.) at 0° for 3½ hr., and the solvent was removed under pressure. The ozonide was then warmed with water (50 ml.) for 15 min. Next day the solid residue was collected and purified from methanol to yield ergoflavin (0·6 g.). The residual aqueous hydrolysate was saturated with sodium chloride and extracted with ethyl acetate. Evaporation

of the dried extract gave a yellow glass. A solution of this was chromatographed on a column (15 × 2·5 cm.) of silica and eluted with chloroform. Evaporation of the eluate furnished a yellow residue which, on crystallisation from ether, gave 2-*carboxyhemiergoflavin* in needles (0·15 g.), m. p. 272° (decomp.), $[\alpha]_D^{20}$ +6·6 (*c* 10·1 in acetone), $\lambda_{max.}$ 233, 260, and 342 mμ (log ε 4·24, 3·97, and 3·60) (Found: C, 55·5; H, 4·0. $C_{16}H_{14}O_9$ requires C, 54·9; H, 4·0%). This compound occludes ether of crystallisation very tenaciously, exhibits an intense plum-red ferric reaction in alcohol, dissolves readily in aqueous sodium hydrogen carbonate with effervescence, and does not react with Brady's reagent. Methylation of 2-carboxyhemiergoflavin (150 mg.) in boiling acetone (30 ml.) containing dimethyl sulphate (0·3 g.) and potassium carbonate (1·5 g.) during 3½ hr. gave 2-*methoxycarbonyl*-1,9-*di*-O-*methylhemiergoflavin* in needles (75 mg.), m. p. 58·2 [Found: C, 58·2; H, 4·7; OMe, 23·8. $C_{16}H_{11}O_6(OMe)_3$ requires C, 58·2; H, 5·1; OMe, 23·6%]. This ester has a negative ferric reaction in alcohol.

When refluxed in water (15 ml.) for 21 hr. a suspension of 2-carboxyhemiergoflavin (0·20 g.) gradually dissolved, to be replaced later by a crystalline solid which separated from the hot solution. This solid product was purified from aqueous methanol to yield *hemiergoflavin* (0·17 g.) in clusters of pale yellow needles, m. p. 228° $[\alpha]_D^{20}$ +113° (*c* 5·5 in methanol), $\lambda_{max.}$ 282 and 370 mμ (log ε 3·94 and 3·39) (Found: C, 58·4; H, 4·9. $C_{15}H_{14}O_7$ requires C, 58·8; H, 4·6%). This compound exhibits an intense green ferric reaction in alcohol. Methylation of hemiergoflavin (105 mg.) by the dimethyl sulphate–potassium carbonate method in boiling acetone (30 ml.) during 1½ hr. gave 1,9-*di*-O-*methylhemiergoflavin* which formed rods (110 mg.), m. p. 277° (from acetone–methanol), having a negative ferric reaction in alcohol, $[\alpha]_D^{20}$ +93·4 (*c* 2·785 in $CHCl_3$) [Found: C, 60·4; H, 5·4; OMe, 18·6. $C_{15}H_{12}O_5(OMe)_2$ requires C, 61·1; H, 5·4; OMe, 18·6%].

Oxidation of di-*O*-methylhemiergoflavin (70 mg.) with the Jones reagent [8] in the usual way gave *di*-O-*methylhemiergoflavinone* (53 mg.) which formed needles, m. p. 202—204° (from methanol) (Found: C, 61·2; H, 5·2. $C_{17}H_{16}O_7$ requires C, 61·4; H, 4·9%).

A mixture of hemiergoflavin (0·9 g.), iodine (0·3 g.), iodic acid (0·1 g.), and ethanol (15 ml.) was stirred at room temperature for 3 hr. The pale yellow solid which separated was purified from methanol to yield 4-*iodohemiergoflavin* (0·3 g.) in yellow needles, m. p. 270—272° (decomp.) (Found: C, 41·4; H, 3·0; I, 29·4. $C_{15}H_{13}IO_7$ requires C, 41·7; H, 3·0; I, 29·4%).

Methylation of this iodo-compound by the dimethyl sulphate–potassium carbonate method in boiling acetone during 1½ hr. gave (quantitatively) 4-*iodo*-1,9-*di*-O-*methylhemiergoflavin* in prisms, m. p. 227° (from acetone–methanol) (Found: C, 44·5; H, 3·6; I, 27·8. $C_{17}H_{17}IO_7$ requires C, 44·4; H, 3·7; I, 27·6%).

Demethylation of Tetra-O-*methylergoflavol.*—A solution of tetra-*O*-methylergoflavol (0·25 g.) in acetic acid (20 ml.) and concentrated hydrochloric acid (5 ml.) was refluxed for 1½ hr. Removal of the solvent *in vacuo* gave a glass which was purified from aqueous acetic acid or chloroform–light petroleum to yield a microcrystalline *solid* (0·2 g.), m. p. 184° [Found: C, 58·8; H, 7·5; OMe, 10·6. $C_{30}H_{32}O_{12}(OMe)_2$ requires C, 59·3; H, 6·0; OMe, 9·6%].

9,9′-*Di*-O-*methyl*-4,4′-*dinitroergoflavin.*—Nitration of di-*O*-methylergoflavin (0·5 g.) dissolved in concentrated nitric acid (30 ml.) occurred during 25 min., to yield 9,9′-*di*-O-*methyl*-4,4′-*dinitroergoflavin* as yellow prisms, m. p. 300° (from acetone–methanol) (Found: N, 3·8. $C_{32}H_{28}N_2O_{18}$ requires N, 3·9%).

4,4′-*Diaminoergoflavin.*—Reduction of 4,4′-dinitroergoflavin (0·5 g.), dissolved in ethyl acetate (50 ml.) containing 10% palladium–charcoal (0·1 g.), occurred during 4 hr., to yield 4,4′-*diaminoergoflavin* which separated from chloroform–light petroleum in bright red needles (0·3 g.), m. p. 340° (Found: C, 57·9; H, 5·6; N, 5·8. $C_{30}H_{28}N_2O_{14}$ requires C, 56·7; H, 4·4%).

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