SYNTHESIS OF A ^{125}I-LABELLED DERIVATIVE OF THE ANTIBIOTIC GRISEOFULVIN

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A derivative of griseofulvin has been synthesised, in which the 2′-O-methyl group is replaced by a 2′-(2-iodoethoxy), ^{125}I-labelled group. This derivative is at least as potent as griseofulvin itself, when assayed for inhibition of growth on the Myxomycete *Physarum polycephalum*.

Griseofulvin, a substance produced by some fungi of the genus *Penicillium*[1], is used in medical and veterinary practice against fungal skin diseases[1,2] and occasionally in rheumatology.[3] Although a very large number of modified derivatives of griseofulvin have been synthesised[4], the mode of action of this antibiotic is poorly understood. One of the targets of griseofulvin, at the cellular level, seems to be the microtubular system[5], especially the mitotic apparatus[6]. These indications are in agreement with some preliminary *in vitro* studies[7]. The main problem encountered in the *in vitro* experiments is the absence of an easily made, clean radioactive compound of high specific activity, although three radiolabelled derivatives have already been prepared. Tritiated griseofulvin (^{3}H-4 methoxy, 4.8 mC/mM) has been used[8] but its preparation has not been described. Griseofulvin labelled either with ^{14}C[9] or with ^{36}Cl[10] (2μC/mM) were obtained through cultivation of *Penicillium griseofulvum*. In order to fulfill all the previous requirements, we proceeded to the synthesis of a griseofulvin analog which can be labelled with radioactive iodine at the last step of the synthesis.

Fig. 1. Structure of griseofulvin: (2*S*, 6′*R*)-7-chloro-2′,4,6-trimethoxy-6′-methylspiro-[benzofurano-2(3H), 1′(2)cyclohexene]-3,4′-dione.

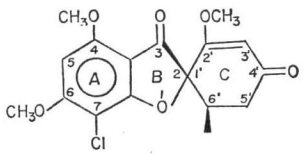
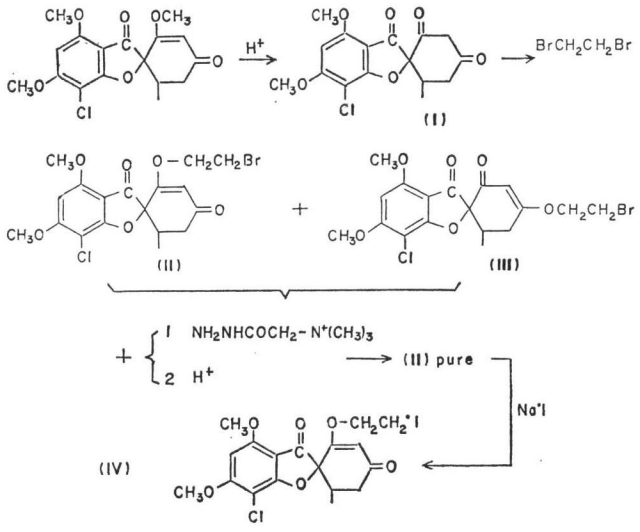


Fig. 2 Flowsheet of synthesis.



Methods

Apparatus: NMR spectra were run on a Varian EM360 spectrometer operating at 60 MHz with TMS as internal standard ($CDCl_3$ solutions). Infrared spectra were obtained in CCl_4 on a Beckman IR-10 apparatus, and mass spectra with a Varian MAT 311 spectrometer. Melting points were measured in open capillaries and are not corrected.

Chemicals: Griseofulvin was a gift of Imperial Chemical Industries, and $Na^{125}I$ was bought from Amersham (solution in NaOH, 100 mC/ml).

Synthesis of Unlabelled Material (Fig. 2):

For hydrolysis of griseofulvin to griseofulvic acid (**I**), 50 g were dissolved in 250 ml acetic acid, and 50 ml of 2 N H_2SO_4 were added[11]. The mixture was treated for 45 minutes in a water bath. After cooling, the precipitate was collected, washed with methanol and ether, and dried (yield of **I**, 90%).

The synthesis proceeds *via* O-alkylation of the enolate anion. The pitfall of the reaction as described in ref. 12 is the occurrence of O-alkylation at 2′- and 4′- on ring *C*. We therefore decided to separate the isomers while still working on cold material and to provide an opportunity for labelling at the last step.

A mixture of 30 g **I** and 105 g dry Na_2CO_3 was suspended in 700 ml anhydrous acetone and refluxed during 25 hours, with gradual addition of 60 ml dibromoethane. The cooled mixture was filtered, and the precipitate washed with hot acetone. The combined filtrate and washings were evaporated under reduced pressure. The residue was extracted with benzene - ethyl acetate (8: 2), washed with water, dried on molecular sieves and evaporated. Yield: 40 g (61%) of **II** + **III**.

Separation of the 2′- and 4′-Isomers[12]:

The product was taken up in 180 ml ethanol, 20 g GIRARD's P reagent and 20 ml acetic acid added, and the mixture refluxed for 45 minutes. It was then poured in a solution of 30 g $NaHCO_3$ in 500 ml H_2O, and extracted with 4 × 200 ml ethyl acetate. This procedure eliminates most of the 4′-isomer which does not form a GIRARD compound under these conditions. The aqueous layer was acidified with 2 N HCl, and 500 ml ethyl acetate added. After 4 hours of mechanical shaking, the upper layer was decanted, washed with water, dried and evaporated. Yield of **II**: 7.4 g.

Purification of **II**:

The raw material was taken up in benzene, refluxed with charcoal and chromatographed on a column of neutral alumina with benzene - ethyl acetate (85: 15). The purity was checked by thin-layer chromatography on silica gel, with a 3: 2: 1 (v-v) mixture of benzene, chloroform and ethanol as solvent. The product (4 g, 10% from the crude mixture of isomers) melted at 80°C; the m.p. was not modified by further crystallizations in CCl_4.

Synthesis of the Iodinated Analog (**IV**):

One g of **II**, 0.375 g dry NaI and 75 ml anhydrous acetone were refluxed for 8 hours. The solvent was removed, 200 ml water added and the precipitate collected on a sintered glass funnel. It was dissolved in $CHCl_3$, washed with 2 × 100 ml water and dried on molecular sieves. After removal of solvent, the yield was 0.7 g (63%) of **IV**: (2*S*,6′*R*)-7-chloro-2′-(2-iodoethoxy)-4,6-dimethoxy-6′-methyl-spiro-[benzofurano-2(3H), 1′(2)cyclohexane]-3,4′-dione.

Proofs of Structure:

(**1**) Infrared spectra: The 3- and 4′- keto groups of **II** show up respectively at 1715 and 1675 cm^{-1}, exactly as in griseofulvin or its 2′-enol ethers analogs. The 2′-3′ C=C bond is found in both cases at 1590 cm^{-1}.[13,14]

(**2**) Mass spectrometry: The field desorption mode was used to avoid loss of aliphatic halogens under electron impact. A 10 mg/ml methanolic solution of **II** or **IV** was used. Conditions were: emitter current 11 mA, chamber temperature 120°C, acceleration 3 kV, extraction 6 kV. Molecular peaks appear respectively at masses 444 and 492. Not more than 10% of the bromo analog remains in the iodinated derivative.

(**3**) Proton magnetic resonance: A characteristic feature of the 2′-enol ethers of the grisan series is exemplified in Fig. 3, showing the up-field shift of the 2′-O-alkyl protons compared to the 4′-isomers[15], owing to the shielding effect of the aromatic *A* ring.

Fig. 3. ^{1}H NMR spectra of: (lower trace) 4′-isomer of: (upper trace) the 2′-(2-iodoethoxy)- derivative of griseofulvin.

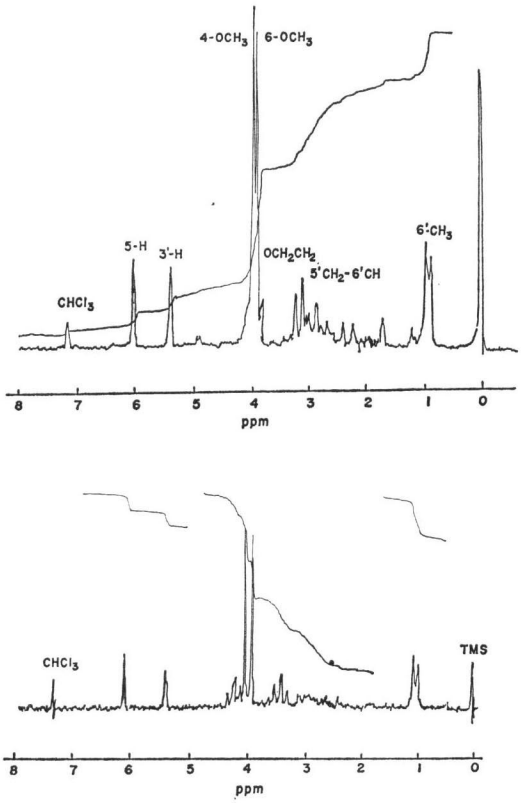
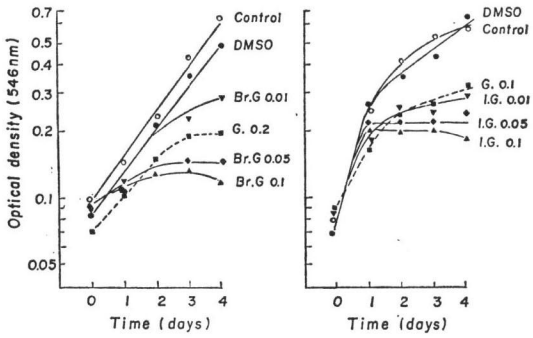


Fig. 4. Biological activity of the synthesised compounds.

Griseofulvin, its bromo derivative and iodo derivative were dissolved in dimethylsulphoxide (DMSO) and added to the minimal defined medium DM_1[17] prior to inoculation by *Physarum* microplasmodia (*P. polycephalum*, strain Cl, ref. 18). Except in the control without DMSO, in all vials DMSO was kept to a final concentration of 1%. Growth was followed by measuring the optical density at 546 nm. In the conditions of culture used (27°C, shaking of 110 strokes per minute, 150 ml of medium per 500-ml vials), with the Cl strain, the O.D. is proportional to the amount of protein in the culture, and 1 unit of O.D. is equivalent to 0.35 mg of protein per ml. All concentrations are expressed in mMoles. G: griseofulvin; Br. G: bromo derivative of griseofulvin; I.G.: iodo derivative of griseofulvin.



Radioactive Synthesis of **IV**:

It was carried out on a 1 mM scale, adding 2 mC of $Na^{125}I$ to 0.444 g of **II** and 0.17 g NaI. The synthesis proceeded as described above, except that the filtration step was omitted. The yield was 0.4 g (specific activity 0.48 mC/mM). The radiochemical purity was checked by TLC as formerly described. It should be noted that the specific activity can easily be increased if necessary.

Biological Activity of the Compounds Synthesised

The action of the bromo and iodo derivatives of griseofulvin which have been synthesised was checked on a fungal model system: *Physarum polycephalum* (Myxomycete). *Physarum* shows two main forms during its biological cycle: uninucleate amoeba which are insensitive to the action of griseofulvin (M. WRIGHT, unpublished data) and polynucleate plasmodia which are sensitive to griseofulvin[16]. The toxicity of the synthetic derivatives towards *Physarum polycephalum* is slightly greater than the toxicity of griseofulvin (Fig. 4). Although the biological significance of this increase of toxicity is not clear, it is in agreement with the published observations concerning the biological action of some other griseofulvin analogs. First, the presence of a 2′-(2-ethoxy) chain ("R10/32")[4] does not modify the biological activity towards *Botrytis allii*, *Microsporum canis* and *Cercospora melonis*. Second, although the compounds we synthesised have never been described before, it is known that the addition of an halogen in 3′- in conjunction with a 2′-(2-ethoxy) chain (Cl: "R10/126"; Br: "R10/125"; I: "R10/81") enhances their biological activity[4].

The production of metabolites was checked with the radioactive derivative. No difference was found between the extracts made before and after an incubation of 3 days in presence of *Physarum*.

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