formed, but this gas could not be found as evidenced by the lack of infrared absorption at 2950 and 2850 cm.$^{-1}$. Nevertheless, upon standing for several weeks, a $TiCl_4$–H_2 mixture at 90 mm. was observed to react slowly at room temperature to form the brown solid characteristic of the titanium subhalides.

Since the dissociation energy at 25° for the reaction $TiCl_4(g) \rightarrow TiCl_3(g) + Cl$ is only 79 kcal./mole,[5] the excited mercury atoms are capable of producing this dissociation step, as well as $H_2 \rightarrow 2H$. Thus mercury photosensitization of the mixture $TiCl_4 + H_2$ should lead to $TiCl_3(g)$, Cl and H in primary steps. However, Cl undoubtedly reacts with the excess H_2 to form HCl + H and thus Hg*-induced decomposition of $TiCl_4$ also leads to atomic hydrogen formation. The chlorohydride $TiCl_3H$ can be formed by the simple step $TiCl_3 + H \rightarrow TiCl_3H$. Note that reaction between H and $TiCl_4$ would lead to the formation of HCl + $TiCl_3$, furnishing more of the latter as precursor of the chlorohydride. At lower pressures where the concentrations of H are expected to be higher, $TiCl_3H$ can be converted to TiH_4 and higher chlorohydrides by reactions such as

$$TiCl_3H + H \longrightarrow HCl + TiCl_2H$$
$$TiCl_2H + H \longrightarrow TiCl_2H_2, \text{ etc.}$$

At high pressures where the concentrations of H are small by comparison with $TiCl_3(g)$ or $TiCl_2(g)$, it is not surprising that crystalline subhalides form rather than chlorohydrides or TiH_4. Under these conditions $TiCl_3(g) \rightarrow TiCl_3(s)$ and $TiCl_2(g) \rightarrow TiCl_2(s)$ are more probable steps than those leading to hydride formation.

(5) This value was calculated from thermochemical data in the JANAF Thermochemical Tables, Dow Chemical Co., Midland, Michigan, Dec. 31, 1961.

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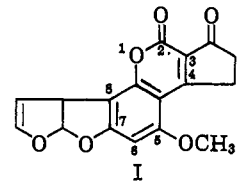
AFLATOXINS B AND G

Sir:

Many outbreaks of an unusual toxicity in several domestic animal species have been reported in Britain.[1] The causative agents have been identified as metabolites of the fungus *Aspergillus flavus* Link ex Fries which infests feed ingredients during harvest or storage.[2] Preliminary reports have dealt with the isolation and characterization of two toxins produced by this mold.[3-5]

This communication is concerned with the isolation and structure elucidation of the two major toxic metabolites. The compounds were isolated from 200 mg. of crude extract generously provided by the U. S. Food and Drug Administration. This concentrate proved to contain substantially greater activity than similar extracts prepared previously in this Laboratory utilizing a different mold variant. The material was prepared as follows. Cultures of *A. flavus* Link ex Fries were grown on sterilized crushed wheat, extracted with chloroform and the toxins precipitated by adding 20 volumes of petroleum ether. Fractionation into individual components was accomplished by thin layer chromatography. The two major components (out of fifteen discernible) appeared as blue-fluorescent and yellow-green fluorescent bands.

The substance exhibiting blue fluorescence, $C_{17}H_{12}O_6$, mol. wt. 312 (mass. spec.)[6] had m.p. 268–269° dec., $[\alpha]_D^{CHCl_3}$ −558°; λ_{max}^{EtOH} 223, 265, 362 mμ (ε 25,600; 13,400; 21,800); $\nu_{max}^{CHCl_3}$ 1760, 1665, 1630, 1600 cm.$^{-1}$. These constants demonstrated identity with the previously described aflatoxin B.[3] Catalytic reduction in ethanol over palladized charcoal was complete after three moles of hydrogen had been absorbed. The resulting product (II) $C_{17}H_{16}O_5$, m.p. 272–274°, mol. wt. 300 (mass spec.); $[\alpha]_D^{CHCl_3}$ −312°; $\nu_{max}^{CHCl_3}$ 1705 (reduced intensity), 1625, 1600 cm.$^{-1}$ gave important information concerning the structure of the toxin. Its ultraviolet spectrum λ_{max}^{EtOH} 255, 264, 332 mμ (ε 8,500; 9,200; 13,900) had a shape identical with that of the synthetic coumarin (III), m.p. 182–184°, but all three maxima in the reduction product were displaced to longer wave lengths by 7 mμ. The changes in molecular composition and infrared absorption accompanying catalytic reduction are explainable if aflatoxin B contains a carbonyl group in a five membered ring and cross conjugated with the α,β-unsaturated lactone function.



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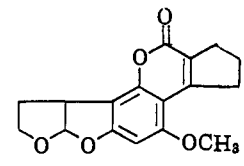
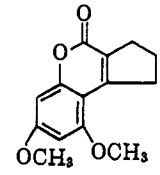
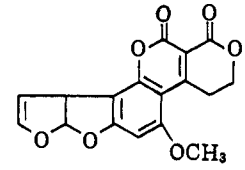


II



III



IV

The nature of the sixth oxygen atom and of the remaining carbon atoms was revealed by the n.m.r. spectrum of aflatoxin B (all in $CDCl_3$; chemical shifts in p.p.m. from tetramethylsilane) which had signals at 6.89 (doublet, J = 7 c.p.s., 1H); 6.52 (triplet, J = 2.5 c.p.s., 1H); 5.53 (triplet, J = 2.5 c.p.s., 1H); 4.81 δ (triplets of doublet, J = 2.5 and 7 c.p.s., 1H). Such a pattern can arise from the four protons of a dihydrofuran ring and agrees with that found in analogous situations.[7] Additional signals at 6.51 (singlet, 1H) and 4.02 δ (singlet, 3H) can be assigned to an aromatic and three methoxy protons while the remaining four protons exhibit A_2B_2 type absorption at 3.42 and 2.61 δ, respectively. The spectrum of the reduction product (II) lacked signals for vinylic protons but the peaks due to the acetal (6.42, doublet, J = 5.5 c.p.s.), aromatic (6.30, singlet) and methoxy protons (3.82 δ, singlet) were still present. Furthermore, the portion of the spectrum representing the six cyclopentane protons is identical in detail with that of the coumarin (III). To complete the argument the attachment of the four carbon side chain to the coumarin ring had to be considered. The n.m.r. spectrum of the synthetic coumarin (III) exhibits aromatic proton signals at 6.35 (doublet, J = 2.5 c.p.s.) and 6.55 δ (doublet, J = 2.5 c.p.s.).

Earlier experience[7] suggests that the high field signals are due to the C_6 proton and because the aro-

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matic proton in the reduction product (II) appears at 6.30 δ aflatoxin B has structure I.

The substance with yellow-green fluorescence, $C_{17}H_{12}O_7$, mol. wt. 328 (mass. spec.), m.p. 244–246° dec., $[\alpha]_D^{CHCl_3}$ −556°; λ_{max}^{EtOH} 243, 257, 264, 362 mμ (ϵ 11,500; 9,900; 10,000; 16,100); $\nu_{max}^{CHCl_3}$ 1760, 1695, 1630, 1595 cm.$^{-1}$ is identical with aflatoxin G.[3] Its n.m.r. spectrum exhibits an A_2X_2 pattern at 4.47 (triplet, J = 6 c.p.s., 2H) and 3.48 δ (triplet, J = 6 c.p.s., 2H). The chemical shifts and multiplicities for all other protons are identical with those of aflatoxin B(I) and we conclude that aflatoxin G has structure IV.

Acute toxicities were determined by biological assay in day-old White Pekin ducklings. Groups of ten animals weighing 51 ± 4 g. received various dose levels of the appropriate compound dissolved in propylene glycol, each animal receiving 0.1 ml. by stomach tube. Mortality and body weights were recorded 48 hr. after administration. Under these conditions the LD_{50} for aflatoxin B (I) was calculated as 28.2 μg. with 95% confidence limits of 24.7 and 32.2 μg. The LD_{50} for aflatoxin G (IV) was estimated to be 90 μg. Administration of 50 μg. of the reduction product (II) resulted in no mortality compared to 100% mortality with the same dose of the parent compound (I).

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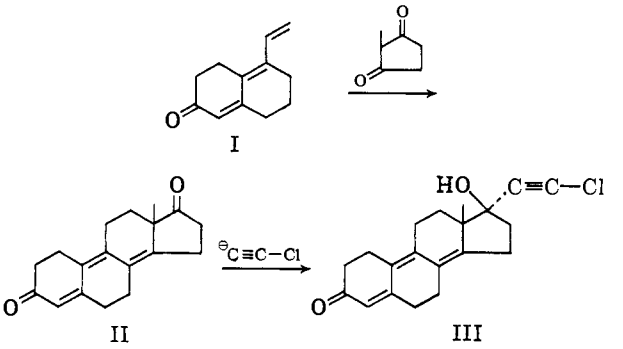
SYNTHESIS OF 19-NOR STEROIDS. II. *d,l*-17α-CHLOROETHYNYL-19-NOR-4,10(9),8(14)-ANDROSTATRIENE-17β-OL-3-ONE[1]

Sir:

It was shown previously[2] that introduction of a C-9, 10 double bond markedly enhances the gonadotrophin inhibiting activity of 17α-chloroethynyl-19-nor-4-androstene-17β-ol-3-one. It was, therefore, of interest to determine whether an additional double bond at C-8,14 would further potentiate activity.

We wish to report the preparation of *d,l*-17α-chloroethynyl-19-nor-4,10(9),8(14)-androstatriene-17β-ol-3-one (III) and its precursor, *d,l*-19-nor-4,10(9),8(14)-androstatriene-3,17-dione (II) by a relatively short synthesis.



Reaction of (I)[3] with 2-methylcyclopentane-1,3-dione[4] in the presence of diethylamine afforded II, m.p. 131–132°; ultraviolet λ_{max}^{MeOH} 350 mμ, ϵ 24,200 (*Anal.* Found: C, 80.90; H, 7.60). It is interesting to note that both Michael condensation and cyclodehydration occurred in the basic condensing medium in *ca.* 17% yield, in view of earlier reports[3,5,6] that in analogous systems acid is required to accomplish the dehydration reaction.

Selective chloroethynylation of II at C-17 was achieved using sodium chloroacetylide in liquid ammonia[7] to yield III, m.p. 159–160°; ultraviolet λ_{max}^{MeOH} 358 mμ, ϵ 19,900 (*Anal.* Found: C, 72.97; H, 6.45).

Preliminary test results[8] with racemic III showed oral gonadotrophin inhibition[9] (parabiotic rats) and oral progestational activity[10] of 1.5–2.0× 17α-ethynyl-19-nor-4-androstene-17β-ol-3-one.[11]

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RECEIVED MARCH 27, 1963

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(2) J. H. Fried, T. S. Bry, A. E. Oberster, R. E. Beyler, T. B. Windholz, J. Hannah, L. H. Sarett and S. L. Steelman, *J. Am. Chem. Soc.*, **83**, 4663 (1961); *cf.* M. Perelman, E. Farkas, E. J. Fornefeld, R. J. Kraay and R T. Rapala, *ibid.*, **82**, 2402 (1960).

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(8) We wish to thank Dr. S. L. Steelman of the Merck Institute for Therapeutic Research for performing these tests.

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(11) In this assay 17α-chloroethynyl-19-nor-4,10(9)-androstadiene-17β-ol-3-one has an activity of 6–8× 17α-ethynyl-19-nor-4-androstene-17β-ol-3-one (ref. 2).