EQUISETIN, AN ANTIBIOTIC FROM *FUSARIUM EQUISETI* NRRL 5537, IDENTIFIED AS A DERIVATIVE OF N-METHYL-2,4-PYROLLIDONE

Sir:

Various species of *Fusarium* produce toxins that are implicated in mycotoxicoses in several areas around the world. During a survey of this genus, we found that one species, *Fusarium equiseti* (CORDA) SACCARDO, produced an antibiotic in yields of 5 g/kg when grown on corn grit medium at room temperature (20~24°C). Production, isolation and biological activity of the antibiotic, assigned the trivial name equisetin, have been reported[1]. Equisetin is active against several strains of Gram-positive bacteria—*Bacillus subtilis*, *Mycobacterium phlei* and *Staphylococcus aureus*—and the Gram-negative bacteria *Neisseria perflava*, at concentrations of 0.5~4.0 μg/ml of growth substrate; however, it did not inhibit other Gram-negative bacteria tested nor fungi. The LD_{50} in mice is 63 mg/kg body weight.

Chemical and spectroscopic evidence indicate equisetin to be an N-methyl tetramic acid (1-methyl-3-acyl-5-hydroxymethyl-2,4-dione) as shown in Fig. 1. The proposed structure is unique among metabolites insofar as it contains a hydroxymethyl group at position C_5 which can form an intramolecular hydrogen bond bridge with the acyl carbonyl group of the β-triketone system.

Elemental analysis and high resolution mass spectroscopy of equisetin (m.p. 65~66°C), as well as microanalysis of its copper salt and tetrahydro derivative gave a molecular composition of $C_{22}H_{31}NO_4$. The UV spectrum of equisetin with characteristic pH-dependent shifts, its IR spectrum (Table 1), formation of its copper salt and positive $FeCl_3$ and $TiCl_3$ tests are similar to those given by the acyl tetramic acid, tenuazonic acid[2] and the N-methyl tetramic acid, decahydroerythroskyrine[3].

Presence of a hydroxymethyl group at C_5 and an N-methyl lactam in the β-triketone structure was indicated by NMR, mass spectroscopy and chemical transformations. The 170.0453 frag-

Fig. 1.

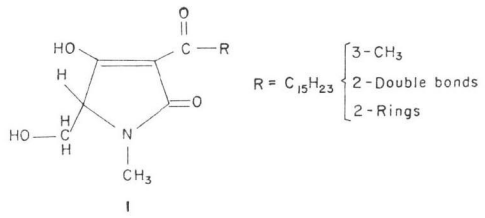

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Fig. 2.

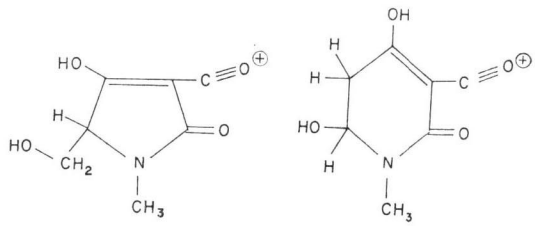

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Table 1. Spectral absorption of equisetin, tenuazonic acid, and decahydroerythroskyrine

Compound	UV maxima						IR($CHCl_3$)
	EtOH		Base		Acid		
	λ	ϵmol	λ	ϵmol	λ	ϵmol	
Equisetin[a]	232 292	6,900 10,000	252 292	8,210 7,400	234 294	5,680 8,860	1,680, 1,650, 1,560 cm^{-1}
Tenuazonic acid[b]	217 277	5,240 13,400	240 279	11,750 14,700	220 277	6,310 12,600	1,735, 1,705, 1,674, 1,630 cm^{-1}
Decahydroerythro-skyrine[c]	225 284	7,250 10,990	246 228	13,800 13,800			1,710, 1,690, 1,635, 1,615 cm^{-1}

a) Base; 0.01 N KOH-EtOH; acid, 0.05 N H_2SO_4-EtOH.
b) See reference 2.
c) See reference 3.

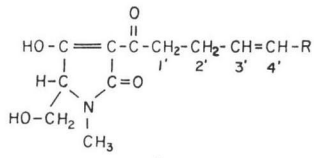
ment in the mass spectrum of equisetin corresponds to $C_7H_8NO_4$. Since this fragment ion contains the nitrogen and all the oxygen of equisetin, it must consist of moiety **2** or **3** as shown in Fig. 2. Reduction with bis-(2-methoxyethoxy)-aluminum hydride (Red-Al, Aldrich Chemical Co.) results in a cyclic amine as evidenced by the characteristic shift of the NMR N-methyl signal from δ 3.03 to δ 2.43[3]. The ease of dehydration, which occurs when equisetin is treated with either acetic anhydride in pyridine or *p*-toluenesulfonic acid in boiling benzene, is consistent with a hydroxy group β to a carbonyl function.

High resolution mass analysis of the dehydrated product gave a molecular ion peak corresponding to $C_{22}H_{29}NO_3$ and a major fragment ion corresponding to $C_7H_6NO_3$. NMR signals at δ 4.58 and δ 4.74 (J_{gem} 2 Hz) are in agreement with a terminal vinyl methylene, which comes from a hydroxymethyl group in moiety **2**. The hydroxyl group of moiety **3** would result in a *cis* double bond, which would emit NMR signals similar to those given by the antibiotic mocimycin, a substituted 4-hydroxy-3-propionyl-2(1H)-pyridone[4]. This evidence disproves existence of moiety **3**. In addition, the NMR spectrum of equisetin in $CDCl_3$ exhibits an A_2B pattern of the type $R_2CH\text{-}CH_2R$ in which the methylene protons appear as a doublet at δ 3.0 and the methine proton as a triplet at δ 3.58 (J 4 Hz). The same protons in deuterated benzene appear as an ABC pattern; (A δ 3.7, B δ 3.06, and J_{AB} 12 Hz, $J_{AC}=J_{BC}$ 4 Hz).

Irradiation of the methine proton at δ 3.06 gave an AB quartet centered at δ 3.6. Molecular models of equisetin indicate that a hydrogen bond could easily be formed between the C_5 hydroxymethyl group and the acyl carbonyl but not the other carbonyls. The hydrogen bond could account for our inability to find an NMR signal attributable to a D_2O exchangeable proton. This is further verified by the consumption of 2 moles of sodium periodate by equisetin as determined by method of AVIGAD[5].

The mass spectral fragmentation pattern of equisetin indicates the R group of **1** to be $C_{15}H_{23}$. The molecular formula of equisetin implies that the compound must contain, in addition to the

Fig. 3.



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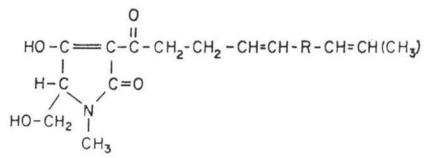

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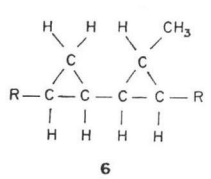
β-triketone ring, four double-bond equivalents (DBE). Consumption of 2 moles of hydrogen (product molecular ion $C_{22}H_{35}NO_4$, major fragment in $C_7H_8NO_4$) either over platinum oxide or 5% palladium-on-charcoal in ethanol indicates two reducible functions. One of these, an olefinic moiety of the type $RCCH=CHCH_3$ (δ 1.46, J 4 Hz, C_6D_6) was established by spin decoupling experiments (irradiation of olefinic protons at δ 5.40 collapses the olefinic methyl signals at δ 1.46) and absence of allylic coupling. The other reducible group presumably also involves a double bond because the protons in the vinyl region (δ 5.2~5.4) disappear on hydrogenation. The mass spectra of hydrogenated equisetin showed a series of fragments at *m/e* 170 ($C_7H_8NO_4$), *m/e* 185 ($C_8H_{11}NO_4$), *m/e* 199 ($C_9H_{13}NO_4$) [which were common also to equisetin] and two new fragments at *m/e* 213 ($C_{10}H_{15}NO_4$), and *m/e* 227 ($C_{11}H_{17}NO_4$) indicating the formation of two methylene groups. The two fragments at *m/e* 213 and *m/e* 227 of hydrogenated equisetin suggests the location of an olefinic bond at C_3' and C_4' as shown in formula **4** (Fig. 3). Also, the fragment at *m/e* 334 ($C_{19}H_{28}NO_4$) would suggest a loss of a propyl group (C_3H_7) from the fragment *m/e* 377 ($C_{22}H_{35}NO_4$) which is in agreement for the terminal methyl olefinic moiety ($RCH=CHCH_3$) shown by NMR. Hence, the equisetin structure could be extended to the formula **5** shown in Fig. 3.

The remaining two DBE must then be attributed to ring structures. Methyl signals at δ 0.90,

Fig. 4.



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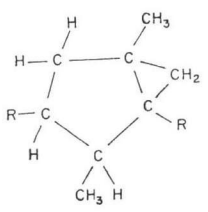

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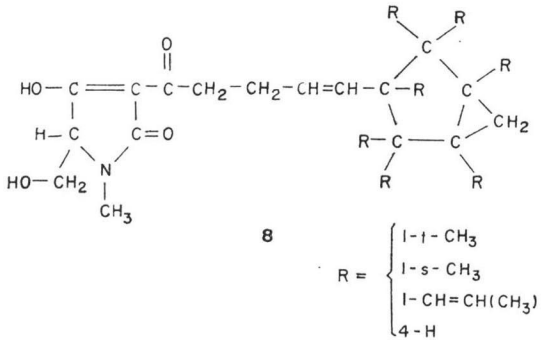
Fig. 5.



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R = 1-t-CH_3, 1-s-CH_3, 1-CH=CH(CH_3), 4-H

6 Hz and δ 1.41 in the NMR spectrum ($CDCl_3$) of equisetin were attributed to secondary and quaternary methyl groups, respectively. KUHN-ROTH analysis gives 1.75 moles of acetic acid accounting for an olefinic and a secondary methyl groups. Values for these groups generally found in the literature are 0.85 and 0.95, respectively. The remaining methyl must be quaternary, which does not react in the KUHN-ROTH procedure. Upon hydrogenation of equisetin in glacial acetic acid at 60°C, 3 moles of hydrogen were taken up as evidenced by a mass of 379, thus indicating a cyclopropane ring[6]. The NMR of this reduced equisetin product showed two tertiary methyl group signals (δ 1.51, δ 1.54). In view of this evidence, moiety **7** would best support this data because two cyclopropyl rings as shown in moiety **6** (Fig. 4) should yield an additional primary methyl which was not detected in the NMR. This evidence supports the proposed structure **8** (Fig. 5) with the location of the substituents off the fused ring system to be determined.

Periodate oxidation of the sodium salt of equisetin gave a compound (m.p. 111~113°C) which chromatographed as a single spot on SiO_2 TLC. It was analyzed as its methyl ester by mass spectroscopy and gave a molecular ion of 419, which corresponds to a formula of $C_{23}H_{33}NO_6$. The mass spectral fragmentation pattern also contained prominent ions, which correspond to $C_{16}H_{24}O$, $C_{15}H_{23}$ and $C_7H_{10}NO_5$. The fragmentation is in accord with the proposed structure **8**. The acyltetramic chromophore also has been found in the antibiotics oleficin[7] and streptolydigin[8], which are produced by some *Streptomyces* species.

RONALD F. VESONDER
LARRY W. TJARKS
WILLIAM K. ROHWEDDER
HARLAND R. BURMEISTER
JAMES A. LAUGAL*

Northern Regional Research Center,
Agricultural Research, Science and Education Administration,
U.S. Department of Agriculture,†
Peoria, Illinois 61604, U.S.A.
*Eureka College
Eureka, Illinois 61530, U.S.A.

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