QUADRONE, A NEW ANTITUMOR SUBSTANCE PRODUCED BY *ASPERGILLUS TERREUS*

PRODUCTION, ISOLATION AND PROPERTIES

G. J. CALTON, R. L. RANIERI and M. A. ESPENSHADE

Washington Research Center, W. R. Grace & Co.,
Columbia, MD, U.S.A.

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A new antitumor compound named quadrone was isolated from the culture broth of *Aspergillus terreus* NRRL 11,156. Quadrone was active against KB cells *in vitro*, but did not possess antimicrobial activity.

A new antitumor compound has been isolated from a fermentation broth of a strain of *Aspergillus terreus*. The antitumor compound was originally designated as G-2408 and has subsequently been named quadrone.

This paper deals with the production, isolation and chemical characterization of quadrone. The structure of this compound will be reported elsewhere.

Taxonomy of the Producing Organism

The organism which produces quadrone was obtained from a soil sample collected in Tipperary, Northern Territory, Australia. The organism has been identified as *A. terreus* by comparison of the morphology (Table 1) and colony characteristics (Table 2) with those given by RAPER and

Table 1. Morphology of the organism

Conidial head	Columnar, 25~50 μ×100~175 μ in length, uniform in diameter. Very young heads with 1~5 conidia in a chain are spherical, but become columnar as they near maturity.
Conidiophores	Smooth 4~5 μ×150~200 μ
Vesicles	7~17 μ in diameter and spherical with some showing a slight pyriform contour.
Sterigmata	Primary series are 5~6 μ×2~3 μ with a phialide shape; secondary series are 5~7 μ×2 μ
Conidia	Globose with a smooth wall and are 2~2.5 μ in diameter.

Table 2. Colony characteristics at 25°C after eight days' growth

Medium	Colony size	Color[2)]	Remarks
CZAPEK-DOX	15 mm	White-fawn	Conidiophores immature
Steepwater agar	30~35 mm	Buckskin yellow in reverse	
Potato dextrose agar	25~40 mm	Fawn-buckskin	
SABOURAUD's dextrose agar	50~60 mm	Fawn	
Malt yeast agar	40 mm	Fawn	

FENNEL[1] for the type species and with a number of *A. terreus* from the American Type Culture Collection. The specific strain has been placed on file with the ARS culture collection (Peoria, Ill. U.S.A.) and is designated *A. terreus* NRRL 11,156.

Experimental Assay Procedures

Assay of the cytotoxic activity was determined by established methods using KB cells *in vitro*.[8] The LD_{50} value was determined by intraperitoneal injection in 20 g mice.

Thin-Layer Chromatographic Procedures

Thin-layer chromatograms were run on silica gel G using chloroform - methanol (9: 1, v/v) as the solvent system. KB active components were determined by separation on preparative thin-layers followed by elution with methanol, and detection in the KB assay. Visualization was carried out with a *p*-anisaldehyde, sulfuric acid, acetic acid and ethanol (5: 5: 1: 90) spray followed by heating at 110°C until maximum color development occurred. Quadrone developes as a light yellow spot.

Spectroscopic Methods

Proton magnetic resonance spectra were recorded on a Varian H-100 spectrometer. Quadrone was solubilized in $CDCl_3$ (approximately 200 mg/ml). Carbon magnetic resonance spectra were recorded on a JEOL PFT 100, equipped with a Nicolet data system.* NMR and CMR chemical shifts are reported as 8 (ppm) units relative to tetramethylsilane.

Complete high resolution mass spectra were obtained on an AEI Model MF30 mass spectrometer equipped with a DS30 data system.**

Fermentation Procedures

Shake Flask Fermentations:

A. terreus NRRL 11,156 was maintained on Potato Dextrose Agar slants (Baltimore Biological Supply, Cockeysville, Md.). A spore suspension was made by the addition of 2 ml of sterilized distilled water to a slant, followed by vigorous agitation of the water over the slant surface with a sterile loop. One milliliter of this suspension was used for the inoculation of a 500-ml Erlenmeyer containing 300 ml of the following medium: 4% glucose, 0.3% Pharmamedia (Trader's Oil Mill Co., Fort Worth, Texas, U.S.A.), 0.1% soybean meal (Southern States Cooperative, Balt., Md., U.S.A.), 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 1% $CaCO_3$, 0.05% NaCl, (All salts were from J. T. Baker & Co., Phillipsburg, N.J., U.S.A.), adjusted to pH 7.1. The inoculated fermentation medium was incubated at 25°C on a rotary shaker at 200 rpm for a period of seven days to obtain quadrone.

Stir Jar Fermentations:

A. terreus was grown as above for a period of 72 hours and used as an inoculum at the rate of 10% (v/v) in a medium consisting of 4% glucose, 0.3% Pharmamedia, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 1% $CaCO_3$, 0.05% NaCl, and 0.15% Pleuronic PL 61 (BASF Wyandotte, Corp., Wyandotte, Michigan, U.S.A.) in deionized water. The fermentor was stirred at

* The CMR spectra were provided by Dr. BARRY SHAPIRO, Texas A & M University, College Station Texas, U.S.A.

** The high resolution mass spectroscopy was carried out by Shrader Analytical Labs, Inc., Detroit, Michigan, U.S.A.

350 rpm, and air was supplied at the rate of 2 liters/minute. The fermentor was maintained at 25°C, and the broth was harvested after 7 days.

Isolation of Quadrone

Sixty liters of broth inoculated with *A. terreus* NRRL 11,156 was fermented as above, and the broth was harvested after 7 days. The broth was filtered, and the filtrate was extracted with *n*-butanol four times (1: 4, *n*-butanol - broth, v/v). The *n*-butanol was removed at reduced pressure in a thin-film evaporator. The resulting syrup was triturated with chloroform and the chloroform was removed by thin-film evaporation at reduced pressure. A small portion of the resulting syrup was used for thin-layer chromatography, and it was determined by bioassay that quadrone had an Rf of 0.67 in chloroform - methanol (9: 1, v/v). From preparative thin-layer chromatography a small amount of crystalline material was obtained, and this was used for seeding the chloroform-soluble material. Upon standing overnight at 4°C, quadrone crystallized. After recrystallization from methanol-water, 12.5 g of quadrone, melting point 185~186°C, was obtained.

Chemical Characterization

Quadrone has a molecular weight of 248.1418 as determined by high resolution mass spectrometry. This corresponds to a molecular formula of $C_{15}H_{20}O_3$ (calculated 248.1412). A bar graph of the low

Fig. 1. Low resolution mass spectrum of quadrone

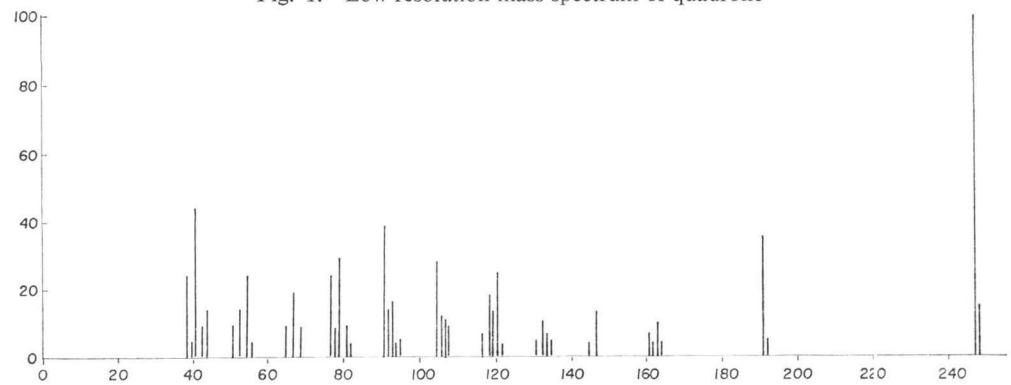


Fig. 2. Infrared spectrum of quadrone (KBr pellet)

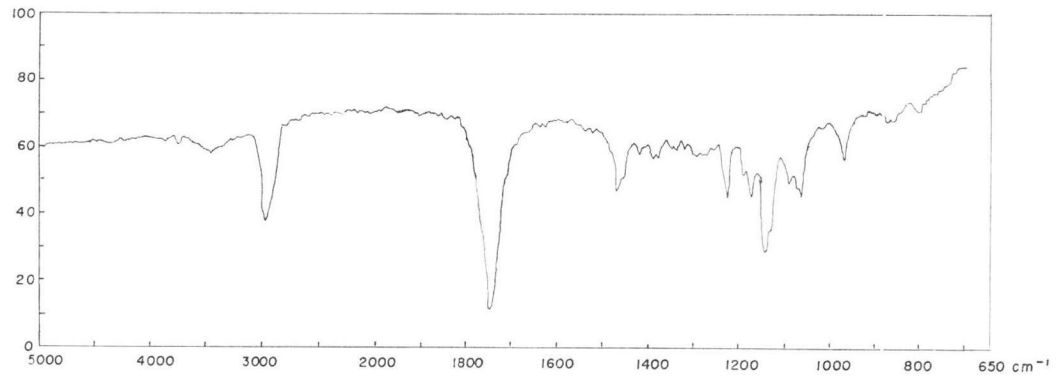
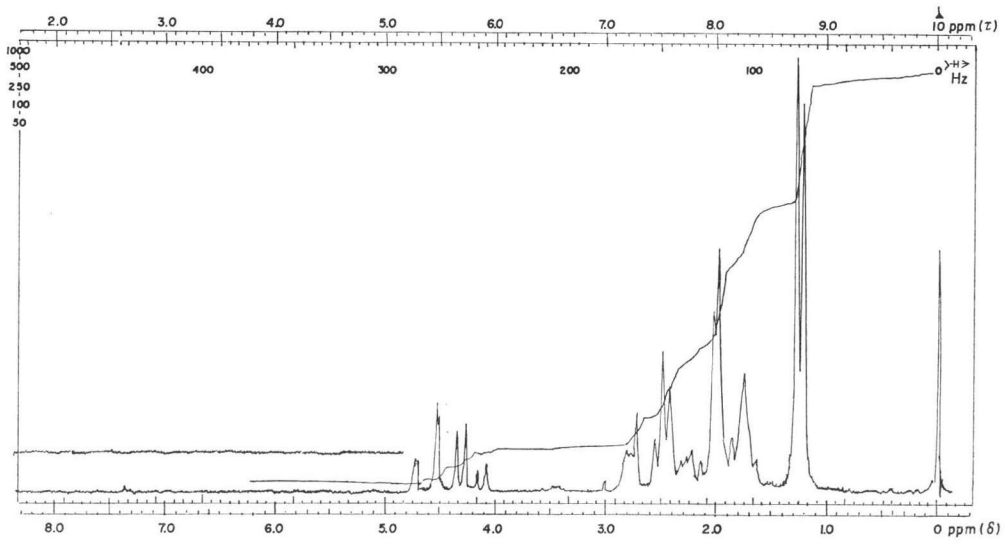


Fig. 3. Proton NMR spectrum of quadrone ($CDCl_3$)



resolution mass spectrum is shown in Fig. 1.

The IR spectrum (Fig. 2) shows absorptions at 1745 cm^{-1} indicating the presence of a five-membered ketone or a δ-lactone and at 1390 and 1375 cm^{-1} indicating the presence of a geminal dimethyl group. The UV spectrum of quadrone showed a weak absorption at 280 nm.

The proton NMR spectrum (Fig. 3) confirmed the presence of a geminal dimethyl group with two, three-proton singlets at δ 1.24 and δ 1.28. The two, one proton doublet of doublets centered at δ 4.23 (J=12, 5Hz) and δ 4.65 (J=12, 1Hz), are characteristic of non-equivalent methylene protons comprising part of a δ-lactone ring.

Absorptions observed in the CMR spectrum and their tentative assignments are shown in Table 3.

Table 3. Carbon-13 NMR chemical shifts and assignment

Chemical shift	Assignment
19.23	Methylene carbon
26.85	Methyl carbon
28.00	Methylene carbon
34.74	Methyl carbon
40.32	Quaternary carbon
43.17	Methylene carbon
45.80	Methine carbon
48.54	Methine carbon
49.69	Quaternary carbon
52.05	Methine carbon
52.21	Methine carbon
52.38	Methylene carbon
65.20	Methylene adjacent to lactone
174.06	Carbonyl carbon
216.52	Carbonyl carbon

Further studies on the structure are underway and will be reported shortly.

Discussion and Results

Biological Activity: The yield of quadrone produced by *A. terreus* NRRL 11,156 was 0.2 mg/ml. The ED_{50} of quadrone (NSC #284437) in the KB assay was 1.3 μg. The intraperitoneal LD_{50} (mouse) value was found to be greater than 340 mg/kg. Quadrone was not found to have antibacterial or antifungal activity at levels of 100 μg/ml or below.

Acknowledgments

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