Pakistan J. Sci. Ind. Res., Vol. 14, No. 6, December 1971

CHEMICAL INVESTIGATION ON THE LENZITES TRABEA

I. Mir, S. Ahmad and A. Hamid

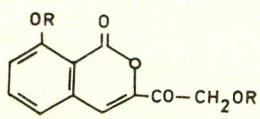
PCSIR Laboratories, Peshawar

(Received October 20, 1970; revised February 1, 1971)

Two compounds have been isolated from *Lenzites trabea* and their structures elucidated by IR,UV and NMR spectroscopy and mass measurements.

The fungus *Lenzites trabea* is a common cause of decay of coniferous trees. McLean *et al.*[1] have recently investigated the mycelium of the fungus and isolated 15-α-hydroxytrametenolic acid from it. This paper describes the isolation and characterization of the compounds from the mycelium and the culture fluid of the fungus *Lenzites trabea*.

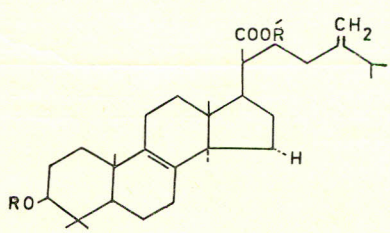
The fungus was cultured on 10% extract of malt-medium containing citric acid and dipotassium hydrogen phosphate. The culture fluid was extracted with ethyl acetate and the extract, after stripping off the solvent, left behind a brown semisolid which on further purification yielded a colourless crystalline compound, m.p. 175°C (I, R = H). From the analysis and mass spectra (M^+, m/e 220), a formula $C_{11}H_8O_5$ was calculated for the compound. This compound gave a violet colour with ferric chloride solution and its IR spectrum showed characteristic absorption peaks at 3450 cm^{-1} (OH), 1720 cm^{-1} (C = O), 1695 cm^{-1} (C = O), 1630 cm^{-1} (C = C), 1578 cm^{-1} and 1600 cm^{-1} (aromatic). Thus the ferric test and IR spectrum indicated the presence of phenolic or enolic hydroxyl function and an aromatic moiety probably in conjugation with a double bond.[2] Treatment of the compound (I, R = H) with pyridine and acetic anhydride gave a colourless crystalline compound (II, R = Ac) for which molecular formula $C_{11}H_6O_5(COCH_3)_2$ was calculated. The IR spectrum of this compound showed absorption peaks at 1780 cm^{-1} (aryl ester), 1750 cm^{-1} (aliphatic ester), 1745 cm^{-1} (C = O), 1630 cm^{-1} (C = C) and 1600 cm^{-1} (aromatic). This clearly showed that the parent compound (I, R = H) contains two hydroxyl groups, one of which is phenolic and the other is alcoholic.[2] The NMR spectrum of the compound (I, R = H) gave a signal at τ 5.35 (2H) s (methylene protons). The aromatic region showed signals at τ 2.9 (1H)q and τ 2.15 (2H)t, forming a typical AMX system. A lower down field proton at τ 0.8 (1H)s could be ascribed to α- or β-CH = in conjugation with a carbonyl and benzene ring.[3] The UV spectrum of the compound showed two absorption bands at λ_{max} 205μ and 335μ. This indicated the presence of α,β-unsaturated ketone in conjugation with a benzene ring.[4] The m.p., IR, UV and NMR spectra of the compound (I, R = H) and its acetyl dervatives (II, R=Ac) were found to be in complete agreement with those of oosponol and diacetyl oosponol[5] respectively.



I, R=H
II, R=Ac

The structure of the oosponol was further confirmed by the mass spectral studies. The spectra showed molecular ion peak, M^+, at m/e 220 and base peak at m/e 189 (M—CH_2OH). Other significant peaks were at m/e 202 (M—H_2O), at m/e 161 (M—CO. CH_2OH), at m/e 133 (M—CH_2 OH—2CO), at m/e 28(CO), at m/e 31 (CH_2OH) and m/e 59 (CO.CH_2OH).

The defatted mycelium of the fungus (petroleum ether) was extracted with alcohol to give a colourless crystalline compound, m.p. 292°C. The compound gave positive Lieberman-Burchard test, indicating it to be terpenic in nature. From the microanalysis and mass spectrum (M^+, m/e 470), a formula $C_{31}H_{50}O_3$ was calculated for it. The IR spectrum of the compound showed absorption peaks at 3450 cm^{-1} (OH), 1725 cm^{-1} (CO_2H) and 890 cm^{-1} (C=CH). On acetylation it gave a monoacetate, and on methylation with dimethyl sulphate it formed a monomethyl ester. This confirmed that the natural product contains one alcoholic hydroxyl group and one carboxylic group. The UV spectrum of the product(III) showed absorption peaks at λ_{max} 230μ and 250μ. The above spectral and analytical data indicated that the natural product is eburicoic acid, a compound earlier isolated from a variety of wood-rotting fungi.[6] The identity of the product(III) was confirmed by comparison of m.p., IR and UV data of its acetate (IV) and its methyl ester (V)



III, R=H,R′=H
IV, R=Ac.,R′=H
V, R=H,R′=Me

with that of acetate and methyl ester of eburicoic acid respectively.

Further confirmation of the structure came from the study of the mass spectra. It showed molecular ion peak, M^+, m/e at 470 and other significant peaks at m/e 452 ($M-H_2O$), at m/e 425 ($M-COOH$), at m/e 407 ($M-COOH-H_2O$), at m/e 427 $[M-CH(CH_3)_2]$, at m/e 315 $[M-CH(COOH)CH_2-CH_2-C(=CH_2)-CH(CH_3)_2]$, at m/e 155 $[CH(COOH)(CH_2-CH_2C(=CH_2)-CH(CH_3)_2]$ at m/e 18 (H_2O), at m/e 45 (COOH), at m/e 63 ($COOH+H_2O$) and m/e 43 $[CH(CH_3)_2]$.

Experimental

All m.ps. are uncorrected. UV absorption spectra were determined with a Beckman spectrophotometer model DK2, IR spectra were taken on a Beckman IR 5 and NMR spectra were recorded on varian A-100 analytical spectrometer.

Isolation of Compound I (R = H).—*Lenzites trabea* was grown on a 10% extract of malt-medium containing citric acid (0.01 g) and dipotassium hydrogen phosphate (0.02 g) in 50 penicillin flasks, each containing 200 ml of sterilized culture medium. After eight weeks the mycelium was removed by filtration, and the filtrate saturated with sodium chloride and then extracted three times with ethyl acetate (3:1). The ethyl acetate extract was dried (Na_2SO_4), reduced to a small volume and then left aside at room temperature. After 4 days, the brown solid mass was filtered off. Fractional crystallizations from ethyl acetate (charcoal) yielded colourless fine needles (0.4 g), m.p. 157°C. (Found: C, 59.5; H, 3.98 (M^+, m/e 220). $C_{11}H_8O_5$ requires: C, 59.99; H, 3.66% (M^+, m/e 220). ν_{max} 3450, 1720, 1695, 1630, 1578 and 1600 cm^{-1}). λ_{max} 205 and 335μ NMR absorption peaks at τ 5.35 (2H)s, τ 2.9 (IH)q, τ 2.15 (2H)t, and τ 0.8 (IH)s.

Diacetyl Derviative (II, R = Ac).—Compound (I, R = H) (0.2 g), acetic anhydride (10 ml) and pyridine (2 drops) were mixed at room temperature. The compound slowly dissolved to give a colourless solution which was set aside at room temperature for 12 hr and then poured into cold water (100 ml). The nearly colourless solid was filtered off, washed with water and crystallized four times from ethanol to yield the di-*O*-acetyl derviative as fine needles, m.p. 155°C (decomp.) (Found: C, 59.2; H, 4.0. $C_{15}H_{12}O_7$ requires: C, 59.21; H, 4.0%. ν_{max} 1780, 1750, 1745, 1700, 1630, 1560 and 1600 cm^{-1}).

Isolation of Eburicoic Acid from the Mycelium Growth of Lenzites trabea.—The dried mycelium (10 g) of *Lenzites trabea* was extracted with petroleum-ether (40–60°C) and then with ethanol. Removal of alcohol from the extract gave an off-white crystalline solid, which on fractional crystallization yielded com pound III (Eburicoic acid) (0.2 g), m.p. 292°C $[\alpha]_D^{19}+38°$ (C 1.2 in $CHCl_3$). (Found: C, 79.36; H, 10.50 (M^+, m/e 470). $C_{31}H_{50}O_3$ requires: C, 79.10; H, 10.7%. (M^+, m/e 470). ν_{max} 3450, 1725 and 890 cm^{-1}).

Acetyl Eburicoic Acid.—Compound (III) (50 mg), was acetylated in a usual manner to give acetyl eburicoic acid (IV) as white needles, m.p. 255°C.

Methyl Eburicate.—Compound (III) (100 mg), dry acetone (50 ml), anhydrous potassium carbonate (10 g) and dimethyl sulphate (3 ml) were refluxed for 6 hr. The mixture was cooled to room temperature and filtered. Evaporation of the filtrate gave an oil which was dissolved in ether, washed with water and dried ($CaSO_4$). The removal of ether left behind a solid mass which, on crystallization from ethanol, yielded methyl eburicate (V) as fine needles, (0.09g) m.p. 154°C. $[\alpha]_D^{20}+45°$ (C 1.2 in $CHCl_3$).

Acknowledgements.—We thank Prof. W.B. Whalley (School of Pharmacy, London) for microanalysis and Dr. P.V. Demarco (Eli Lilly and Co., U.S.A.) for NMR and mass spectra. We also thank Mr. Mohd Ishaque for technical help during this work.

References

1. W. Lawrie, J. McLean and J. Watson, J. Chem. Soc., 1776 (1967).
2. I. Fleming and D.H. Williams, *Spectroscopic Methods in Organic Chemistry* (Mcgraw-Hill, New York, 1966), p. 61 and 67.
3. I. Yamamoto, K. Nitta and Y. Yamamoto, Agr. Biol. Chem., **27,** 813 (1963).
4. A.L. Wilds, L.W. Beck, W. J. Close, C. Djerassi, A. J. Johnson and C.H. Shrink, J. Am. Chem. Soc., **69,** 1958 (1947).
5. K. Nitta, J. Imai, I. Yamamoto and Y. Yamamoto, Agr. Biol. Chem., **27,** 817 (1963).
6. R.M. Gascoigne, J.S.E. Holker, B. J. Ralph and A. Robertson, J. Chem. Soc., 2346 (1951).

Note: After submission of this paper the isolation of eburicoic acid has recently been reported from *Lenzites trabea*, Phyto chem., **10,** 427 (1971).