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ROTENOIDS FROM *AMORPHA CANESCENS* ROOTS*

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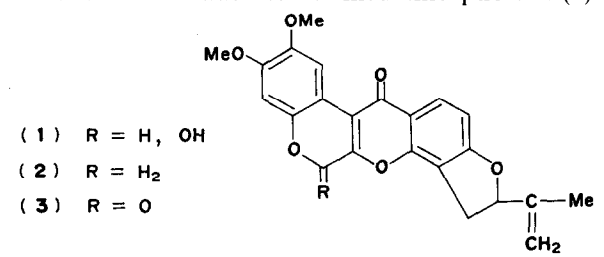
Key Word Index—*Amorpha canescens*; Leguminosae; lead plant; isolation; synthesis; rotenoids; amorpholone; dehydrorotenone; rotenonone; acetylursolic acid.

Abstract—A new rotenoid termed amorpholone has been isolated from a hexane extract of *Amorpha canescens* roots and identified as 6-hydroxydehydrorotenone by spectroscopic methods. Its structure was confirmed by its conversion to rotenonone and by synthesis from rotenone. Also isolated were rotenonone and acetylursolic acid.

INTRODUCTION

Expansion of out natural product program aimed at a chemical investigation of plants common to northern Illinois prompted the exploration of the roots of *Amorpha canescens*, or lead plant, so named from the color of its leaves. Our efforts were further motivated by observations that aqueous alcohol extracts of the roots exhibited anticancer activity in the KB assay. A survey of the literature indicated that no chemical work had been described for this species, but a number of investigations have centered on the rotenoids [1] of *A. fruticosa* (false indigo). Further work also described the isolation of terpenes [2,3]. The present work with *A. canescens* reports the identification of a new rotenoid which has been termed amorpholone (**1**).



(**1**) R = H, OH
(**2**) R = H_2
(**3**) R = O

RESULTS AND DISCUSSION

The rotenoids were obtained in the hexane soluble fraction and initially purified by chromatography on alumina. The first material to be isolated in any substantial quantity was a mixture of sterols with β-sitosterol as the primary constituent. As elution of the column continued, a yellow powdery substance appeared in subsequent fractions. This yellow material was sparingly soluble in chloroform and was resolved into two major components by preparative-TLC.

The less mobile material, amorpholone (**1**) easily crystallized as fine needles and had m.p. 255–260°. An IR spectrum showed an OH absorption at 3260 cm^{-1}, aromaticity at 3000, 1582, 1510, and 900 to 700 cm^{-1} and a highly conjugated ketone at 1630 cm^{-1}. The region between 1450 and 1000 cm^{-1} contained a series of sharp absorptions similar to that from a large, complex molecule like a rotenoid. Identification of amorpholone as a rotenoid was strengthened by its UV spectrum which had maxima at 213, 234, 279, and 305 nm very similar to the maxima shown by dehydrorotenone (211, 236, 281 and 311 nm).

More substantial evidence for the similarity of amorpholone (**1**) to dehydrorotenone (**2**) was gained from a comparison of PMR data. Most of the signals could be readily assigned from data found in the literature for other rotenoids [1,4]. The only signals that did not correlate were a singlet at δ5·08 for two hydrogens at C-6 in the dehydrorotenone spectrum and two doublets at δ7·94 and 6·18 in the amorpholone spectrum. These were eventually assigned to the proton at C-6 and the proton on the C-6 hydroxyl moiety, respectively. The latter assignment was verified by

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the disappearance of the signal from an exchange of the proton with solvent deuterium atoms.

The structure proposed for amorpholone also explains its rather unusual MS. Other than a M^+ at *m/e* 408 the only significant ions were the base peak at *m/e* 391 and a peak at *m/e* 379. The base peak undoubtedly originates by loss of OH from the M^+. This loss (M^+-17) is best explained by the formation of a stable pyrilium ion from the ring to which the OH was attached. On the other hand, if the hemiacetal system of which the OH group is a part opens to the aldehyde form and then loses CHO, the ion *m/e* 379 would result.

The structure for amorpholone was further substantiated by its oxidation to rotenonone (**3**) with chromic acid. The product was easily identifed by the appearance of an IR band at 1747 cm^{-1} characteristic of an aromatic lactone system and a m.p. of 297–299° which was in agreement with that reported [5] for rotenonone. Rotenonone (**3**) later proved to be identical in all respects to the more mobile material obtained after TLC of the column fractions containing amorpholone. Judging by the ease which dehydrorotenone and rotenone are oxidized, it is probable that the occurrence of rotenonone (**3**) is an artefact of aerial oxidation.

As a final proof amorpholone (**1**) was synthesized from rotenone in a 12% overall yield. Rotenone was first hydroxylated by chromic acid to yield rotenolone [6,7]. The ketol was then dehydrated by acid [8] to dehydrorotenone (**2**) which was brominated at C-6 by N-bromosuccinimide. Of the two allylic positions susceptible to bromination, we reasoned the C-6 position might be favored since it was also α to an ether moiety. No attempt was made to isolate the intermediate bromoether owing to ready decomposition. Solvolysis of the resultant α-bromoether with aqueous acetone gave synthetic amorpholone identical in all respects to the natural compound.

The fractions eluted from the column after amorpholone were found to contain acetylursolic acid. Its occurrence is not unusual since it is common to a great variety of plants.

EXPERIMENTAL

All m.ps are uncorrected. IR spectra obtained from the solids incorporated in KBr disks. PMR spectra were recorded in $CDCl_3$ at 60 MHz. UV spectra were measured in BuOH. Si gel HF-254 was employed for all TLC using EtOAc–C_6H_6 (1:3) as solvent.

Isolation procedure. Dried powdered roots (11·5 kg) of *Amorpha canescens* collected in DeKalb and Ogle Counties, Illinois (Northern Illinois University Herbarium Voucher PDS 7365) were exhaustively extracted with hexane in a Soxhlet apparatus. On cooling, the ppt. which formed was removed by filtration, and the filtrate evaporated to 87·9 g. The material was chromatographed on 1·9 kg of neutral alumina, activity I, and 100 × 1. fractions were collected using eluants of increasing polarity.

Sterol mixture. A mixture of sterols was isolated from a fraction (no. 47) eluted by C_6H_6–$CHCl_3$ (1:1) after separation on TLC. Repeated recrystallizations from EtOH afforded material with m.p. 138–145°; ν_{max} 3400, 1640, 1455 cm^{-1}.

Amorpholone. (a) Amorpholone was present in fractions 76–84 eluted with $CHCl_3$–EtOAc (1:1). TLC of 400 mg yielded 35 mg of amorpholone (**1**). Recrystallization from $CHCl_3$ gave 24 mg as fine yellow needles, m.p. 255–260° (decomp.); λ_{max}213 (ϵ 19000), *sh* 234 (13000), 279 (10000), 305 nm (8100); MS 80 eV: *m/e* 408 (M^+), 406, 393, 392, 391 (100), 379, 363, 361; PMR: δ 1·79 (*s*, 3H), 3·30 (*m*, 2H), 3·80 (*s*, 6H), 5·06 (*d*, 2H, *J* 8Hz), 5·54 (*t*, 1H, *J* 8Hz), 6·18 (*d*, 1H, *J* 7Hz), 6·73 (*s*, 1H), 7·09 (*d*, 1H, *J* 9Hz), 7·94 (*d*, 1H, *J* 7Hz), 8·00 (*d*, 1H, *J* 9Hz), 8·50 (*s*, 1H). (b) A mixture of dehydrorotenone (100 mg), *N*-bromosuccinimide (45 mg), and CCl_4 (35 ml) was refluxed for 24 hr. The succinimide was removed, and the filtrate evaporated in a stream of dry N_2. The residue was immediately treated with Me_2CO–H_2O (4:1) for 30 min at 20° then heated at 100° for 1 hr. Removal of the solvent *in vacuo* gave a mixture which was subjected to preparative-TLC. A total of 21 mg of amorpholone (**1**) identical in all respects to the material isolated above was recovered.

Rotenonone (**3**). (a) The column chromatography fractions from which amorpholone was obtained also gave 6 mg of rotenonone after TLC. Recrystallization of the isolated material from MeOH gave bright yellow needles; m.p. 297–299° (Ref. 5, m.p. 298°); ν_{max}1747, 1630, 1600 cm^{-1}. (b) Amorpholone (10 mg) in HOAc (1 ml) was stirred with a 10% soln of Na dichromate in HOAc (1 ml) for 18 hr at 20°. The oxidized material was recovered in Et_2O, washed with H_2O and dried. Removal of the solvent and TLC of the residue afforded 6 mg of rotenonone, identical in all respects with the material above.

Acetylursolic acid. Elution of the alumina column with EtOH–EtOAc (1:19) gave acetylursolic acid in fractions 93–99 as an off-white powder. Two recrystallizations from EtOH yielded colorless crystals with m.p. 245–246° (Ref. 9, m.p. 245°); ν_{max}3225, 1735, 1680, 1260 cm^{-1}; PMR: δ0·75; 0·85, 0·93, 1·13, 2·06, 5·29; MS 80 eV: *m/e* 498 (M^+), 249, 248 (100), 203.

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