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ISOLATION, STRUCTURE AND SYNTHESIS OF 4-HYDROXYISOXAZOLE (TRIUMFEROL), A SEED GERMINATION INHIBITOR FROM AN AFRICAN PLANT

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Summary: The isolation, characterization and an efficient synthesis of 4-hydroxyisoxazole 1, a plant growth regulator isolated from an African plant is reported. It is a new compound, which despite the simple structure, had eluded synthesis.

The leaf extract of the East African folk medicinal plant Triumfetta rhomboidea (Tiliaceae)[1] was found to exhibit antigermination activity against lettuce seeds. Isolation studies were hence monitored by the germination assay which has led to the characterization and synthesis of 4-hydroxyisoxazole 1 or "triumferol", a simple but new compound, as the active principle.

A 50% aqueous methanol extract of 0.5 Kg of the air-dried leaves[2] was concentrated to dryness, and the residue (5.97 g) was extracted with n-hexane and then with ether. Flash chromatography[3] of the bioactive ether extract using a 7:3 v/v mixture of hexane and ethyl acetate or a 5:1 v/v mixture of methylene chloride and ethyl acetate gave 210 mg of the pure active compound, triumferol 1, m.p. 67-68° (from ether/hexane). The lettuce seed antigermination activity of triumferol was 100% at 100ppm and 70% at 50 ppm.

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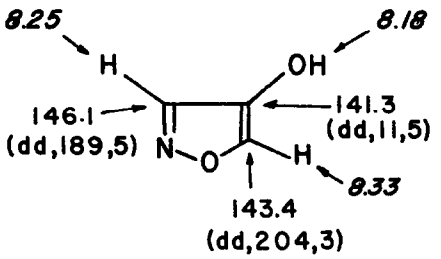


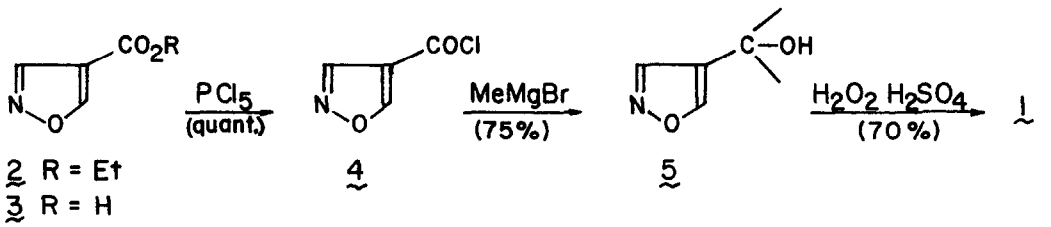
1 ^{1}H-NMR(in italics) and ^{13}C-NMR peaks of triumferol in acetone-d_6

High-resolution EI-MS measurements at room temperature gave the molecular formula of $C_3H_3NO_2$, M^+ 85.0163 (calcd. 85.0163), M^+-HCN peak at 58.0052 (calcd.58.0035). Only three ^{13}C and ^{1}H peaks were present in the NMR spectra (see 1); the 8.18 ppm ^{1}H-signal disappears upon addition of D_2O. Since the three ^{13}C-signals were too closely located for conventional off-resonance decoupling, correlation of the two aromatic ^{1}H-signals with ^{13}C-signals were carried out by the technique of off-resonance gated decoupling.[4] Additional physical constants for triumferol are as follows: IR(CH_2Cl_2) 3560(sh), 3180(br) (conc. dependent OH peaks),1640,1625 (arom. skeletal),1090,1000 cm^{-1}(arom.C-H); UV(MeOH) 240nm(ε 1,100), shifted to 270nm in base; pK_a'(H_2O, from UV),7.75.

These and other data[6] indicated that triumferol was a hydroxylated isoxazole or oxazole, i.e., one of six possible structures. The similarity of the UV spectrum to that of 3,5-dimethyl-4-hydroxyisoxazole (λ_{max} 250nm)[7] and the close chemical shifts of all three ^{13}C-peaks suggested 1 to be the most plausible; i.e., 1 is the only structure in which all three carbons are linked to a single hetero atom. Moreover, 3-hydroxyisoxazole[8], isoxazolin-5-one (5-hydroxyisoxazole)[9] and oxazolin-2-one (2-hydroxyoxazole)[10] are known compounds which differ from triumferol. The fact that triumferol is 4-hydroxyisoxazole 1 was established by the following synthesis.

Although numerous 3- and 3,5-disubstituted-4-hydroxyisoxazoles have been synthesized[11], the parent compound itself singularly has not yet been prepared despite the effort by several groups. In retrospect this is not surprising in view of the fact that all conventional syntheses of substituted 4-hydroxyisoxazole failed (we were benefited by the fact that we knew the compound exists). Thus the reactions of 2-acetoxy-1,3-diones and its various equivalents with hydroxylamine[12] or the metalation of 4-bromoisoxazole[13] led to complex mixtures. Diazo reactions of 4-amino-isoxazole which had been successful in substituted cases[14] only gave water-soluble compounds (1 is only slightly soluble in water). The 1,3-dipolar addition of fulminic acid with vinylidene carbonate[15] did not proceed whereas that with methoxyacetylene

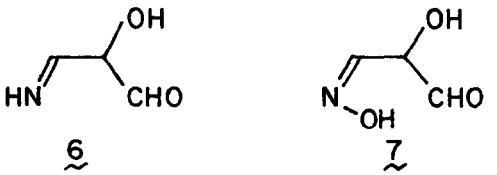
yielded 5-methoxyisoxazole rather than the 4-methoxy derivative. The failure in many of these synthetic attempts is attributable to the base-lability of the protons at C-3 and C-5 of the (potential) 4-hydroxyisoxazole nucleus.



SCHEME 1

The synthesis was finally achieved in satisfactory yields according to Scheme 1. The known ethyl 4-isoxazolecarboxylate 2 was prepared with improved yields in the following sequence. A modified Reformatsky reaction of ethyl orthoformate gave ethyl 3,3-diethoxypropoinate[17], which was converted into ethyl 2,2-diformylacetate[18] by formylation[19] and subsequent acid hydrolysis; reaction of ethyl 2,2-diformylacetate with hydroxylamine yielded ester 2[16] in ca. 50% overall yield from orthoformate. Hydrolysis of 2 with hydrochloric acid gave the known acid 3[16] (quant.) which was treated with 1 equiv. of phosphorous pentachloride. Evaporation of phosphorous oxychloride in vacuo followed by reaction of the residual oil 4[20] with methylmagnesium bromide in ether yielded tert-alcohol 5, oil, 75% yield. A 15 ml dichloromethane solution of 2.83 g of alcohol 5 was treated slowly with an ice-cooled solution of 5 ml of 30% hydrogen peroxide in 6.5 ml of concentrated sulfuric acid at such a rate that a gentle reflux of dichloromethane was maintained.[21] Addition of ice, separation of the organic layer followed by flash chromatography yielded 1.36 g of pure triumferol 1, 70% from alcohol 5.

The occurrence in nature of unsubstituted 4-hydroxyisoxazole, a simple and new bioactive heteroaromatic compound is to be noted. Biogenetically it could arise from the Schiff base 6 (or equivalent) formed from hydroxymalonaldehyde and ammonia, oxidation to its N-oxide, prototropic shift to aldoxime 7, and cyclization; this speculative path is similar to the conventional synthetic routes.[12]



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5. The 8.25 ppm ^{1}H-signal was assigned to 3-H rather than 5-H because it was broader than the 8.33 ppm signal; i.e., the broadness was attributed to the quadrupole effect of the adjacent N atom. Since the ^{1}H and ^{13}C peaks were correlated, these H assignments led to assignments of the ^{13}C peaks.

6. ^{1}H-NMR of acetate(in $CDCl_3$), 8.34(s,3-H), 8.76(s,5-H), 2.28(s,OAc); ^{1}H-NMR of methyl ether(in acetone-d_6), 8.34(s,3-H), 8.50(s,5-H),3.80(s,OMe). In both cases, the broader of the two aromatic peaks was assigned to the 3-H as in the parent compound.[5]

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19. Use of NaH instead of Na^{18} greatly shortened the reaction time.

20. Reaction of 4 with Me_2CuLi in ether gave 4-acetylisoxazole (30% yield), which resisted Bayer-Villiger oxidation.

21. cf. Baumgarten, H.E., Ed. "Organic Syntheses, Coll. Vol. 5;" John Wiley: New York, 1973; p. 818.

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