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STRUCTURE DETERMINATION OF A PHENOLIC PENT-1-EN-4-YNE DERIVATIVE FROM *HYPOXIS ROOPERI**

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Key Word Index—*Hypoxis rooperi*; Hypoxidaceae; pentenyne; hypoxoside; nyasoside; medicinal use.

Abstract—Enzymic hydrolysis of the partially-purified extractives of *Hypoxis rooperi* yielded a minor component identified as 1-(3″,4″-dihydroxyphenyl)-5-(4′-hydroxyphenyl)pent-1-en-4-yne using NMR techniques.

INTRODUCTION

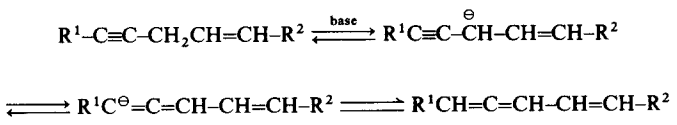
We have previously reported [2] on the isolation and synthesis of compounds from the plant family Hypoxidaceae and referred to their use in traditional African medicine. Parallel to our investigations have been the studies by Marini-Bettolo *et al.* [3–5] on the same plant family. In almost all instances these compounds have had a common pent-1-en-4-yne 'backbone' or some obvious modification of it. Our recent unpublished studies on the metabolism of a wide range of phenolic pent-1-en-4-yne analogues by mammals have shown very clearly that the pattern of phenolic substitution exerts a profound influence on the metabolic pathway that is followed. It is because of these findings that the present naturally-occurring derivative, which has been shown to be 1-(3″,4″-dihydroxyphenyl)-5-(4′-hydroxyphenyl)pent-1-en-4-yne (**1**), is of considerable interest.

RESULTS AND DISCUSSION

Hypoxoside (**2**) was the first pentenyne derivative isolated from natural sources [3, 6] and its aglycone (**3**), named rooperol, has come under close scrutiny on account of its anti-tumour properties [7, 8]. So far the attempts to synthesize rooperol [9] have only met with partial success in that exotic protecting groups, such as *tert*-butyldimethylsilyoxy groups have had to be employed and even in these instances the final deblocking reaction proceeds in unsatisfactory yield. These problems arise from the fact that the pentenyne system in question is very sensitive to the basic conditions used for deprotection and readily rearranges (Scheme 1). We have found that the nature of the phenolic R^1 and R^2 influence profoundly not only the initial coupling reaction leading to the desired pentenyne but they also strongly influence the sensitivity to base in the final stage. Our isolation of compound **1** proved extremely useful since its biological and chemical properties could be compared with those of rooperol. This paper deals largely with a proof of structure for **1** using high-field NMR techniques.

Rooperol (**3**) is obtained by hydrolysis of hypoxoside with β-glucosidase or cellulase [2, 3]. Isolation of hypoxoside from the plant source is readily achieved *via* countercurrent separation of the ethanolic extract, or it can also be obtained in good purity by preparative HPLC separation. From hypoxoside isolated from *Hypoxis rooperi*, and subsequently hydrolysed with one of the above enzymes, we observed that the resultant rooperol (**3**) was contaminated by a very low percentage (*ca* 4%) of two other components of lower polarity as judged by TLC. By repeated separation of rooperol (**3**), using flash chromatography, sufficient contaminating material was isolated and subsequently identified as compound **1**.

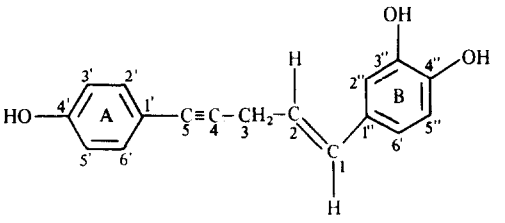
Before dealing with the proof of structure of compound **1** it is of interest to draw attention to the increasing number of compounds being isolated from natural sources in which two phenolic nuclei are linked by a five-carbon bridge containing two positions of unsaturation. Generally it is a pent-1-en-4-yne system. In some instances, as in hinokiresinol (**4**), the linking unit is 'open' with an alkene unit at one end and a phenol moiety at the other. Many of these compounds are biologically active. Apart from rooperol (**3**) [2, 3], nyasol (**4**) [4], hinokiresinol (**4**) [10] and nyasicoside (**5**) [5], a recently isolated compound in this category is the (*Z*,*Z*)-4,4′-(1,4-pentadiene-1,5-diyl)diphenol (**6**) [11] from *Ginkgo biloba*. Subsequently the identical compound has been isolated from the rhizomes of *Alpinia galanga* by Barik *et al.* [12].



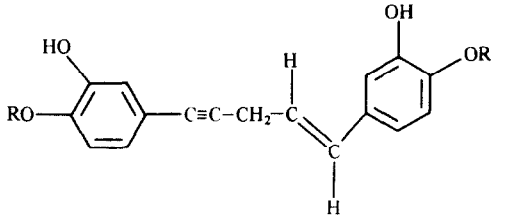
Scheme 1. Rearrangement of pentenyne system under base conditions.

*Part 3 in the series 'Medicinal Plants of Southern Africa'. For part 2, see ref. [1].

†Author to whom correspondence should be addressed.

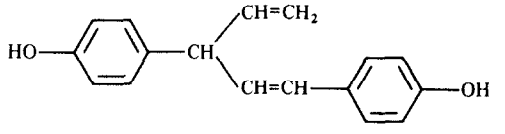


1



2 R = β-D-glucose

3 R = H



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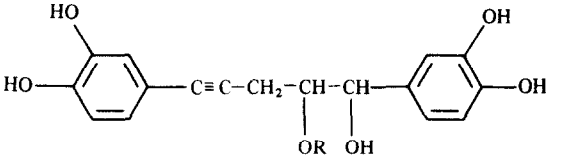
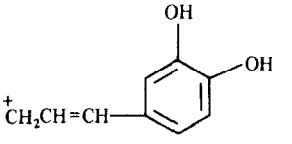


5 R = β-D-glucose



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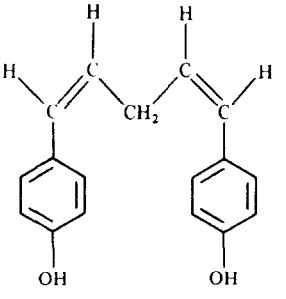

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From its method of isolation compound **1** occurs naturally in the plant as the diglucoside. Its properties in this state are presumably so similar to those of hypoxoside (**2**) (also a diglucoside) that the normal methods of separation were not able to separate the two compounds. However, after hydrolysis, TLC provided a good separation of the tetraphenol derivative (rooperol) from the triphenol derivative **1**. Proof of structure of **1** is based on the following evidence:

(i) An accurate mass determination of the methyl ether gave a value of 308.14054 consistent with a molecular formula of $C_{20}H_{20}O_3$. Loss of a methyl radical gave rise to a prominent peak at *m/z* 293 which is in good agreement with a fragment $C_{19}H_{17}O_3$. The base peak in the mass spectrum was at *m/z* 149 which could readily arise from the fragment **7**, thus providing good evidence that the phenolic ring on the 'alkene side' was catechol. The presence of this pattern of phenolic substitution in one of the aromatic rings can be illustrated by the immediate black colour which develops on a chromatogram sprayed with ammoniacal silver nitrate.

(ii) The ^{13}C NMR analysis of compound **1** provided very strong evidence in favour of the proposed structure. The carbons at C-3, C-4 and C-5 were different from the

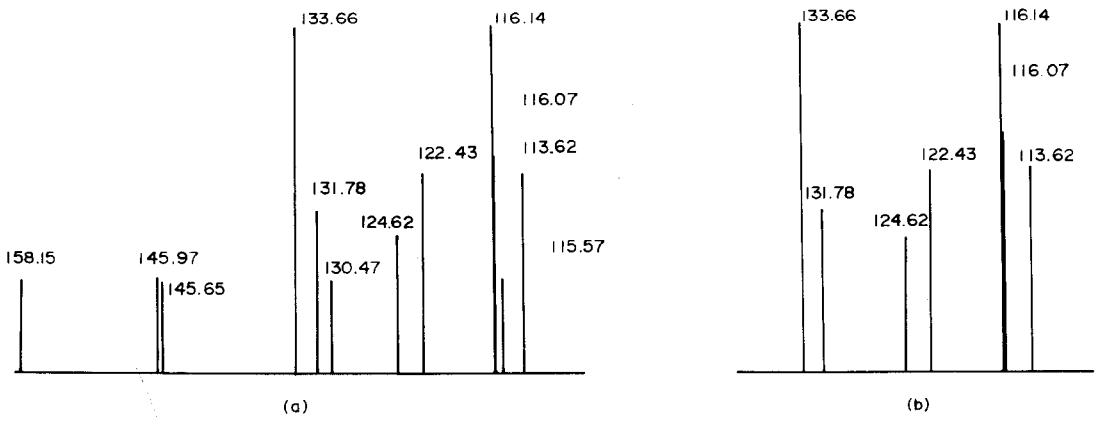

Fig. 1. ^{13}C NMR spectrum (a) and HETCOR spectrum (b) for compound **1**. The aromatic region only is shown.

Table 1. ^{13}C NMR chemical shift data for compounds **1** and **3** [3]

C	**1** in $(CD_3)_2CO$	C	**3** in $(CD_3)_2CO$
1	131.78	1	129.9
2	124.62	2	117.7
3	23.09	3	21.5
4	85.58	4	83.6
5	83.30	5	81.5
1″	130.47	1″	128.8
2″	113.62	2″	111.9
3″	145.65	3″	143.7
4″	145.97	4″	144.0
5″	116.07	5″	114.4
6″	122.43	6″	120.7
1′	115.57	1′	114.2
2′6′	133.66	2′	114.4
3′5′	116.14	3′	143.8
4′	158.15	4′	144.5
		5′	117.4
		6′	122.9

rest and could be identified unambiguously. Three other peaks were prominent and from their downfield position (δ145–158) and their multiplicity (singlets) it was reasonable to assume that they arose from aromatic carbons bearing hydroxyl substituents. This in itself provided good evidence that **1** was indeed a trihydroxy species. At this stage nine carbons, three on the A ring side (2′, 6′ and 3′, 5′ are identical) four on the B ring side (all different) and two on the bridging chain, were unallocated. Analysis of the heteronuclear spin-correlated 2D NMR (HETCORR) spectrum of **1** in conjunction with the ^{13}C NMR shifts allocated to the B ring carbons by Marini-Bettolo [3, 4] revealed the situation shown in Fig. 1. From their position and intensity the peaks at δ133.6 and 116.14 belonged to the 2′, 6′ and 3′, 5′ carbons, respectively. The absence of peaks at δ115.57 and 130.47 in spectrum (b) implied that these were quaternary carbons and belonged to C-1′ and C-1″, respectively. From Marini-Bettolo's earlier work [3] on compound **3** the peaks at δ113.62, 116.07 and 122.43 were assigned to C-2″, C-5″ and C-6″, respectively. Thus only the two peaks at δ124.62 and 131.78 remained unallocated. Examination of the heteronuclear spin-correlated 2D NMR (HETCORR) spectrum showed that the easily discernable protons on C-2 (δ6.08) and C-1 (δ6.62) were coupled to carbons at 124.62 and 131.78, respectively. This then accounted for all the carbons in compound **1** (Table 1).

(iii) The ^1H NMR spectrum (at 80 and 500 MHz) of compound **1** was almost identical to that of **3** with one notable difference. Whereas in the spectrum of **3** the aromatic protons appeared as a complex multiplet resonating between δ6.80–6.95, in the spectrum of **1** there was an uncluttered doublet centred at δ7.32 (J = 8.8 Hz) well separated from the multiplet at 6.78–6.98. A 4-hydroxyl group on ring (A) would give rise to the above pattern with the (2′, 6′) protons resonating at δ7.32. The fact that the protons on C-1, C-2 and C-3 had practically identical chemical shift values to those in compound **3** (see Table 2) is taken as further evidence that the (B) rings in **1** and **3** are 3,4-dihydroxylated.

Table 2. ^1H NMR chemical shift data (ppm) for compounds **1** (at 500 MHz) and **3** (at 80 MHz) in $(CD_3)_2CO$

H	**1**	H	**3**
1	6.62 *dt* (J = 15.73 and 1.51 Hz)	1	6.61 *dt* (J = 15.7 and 1.5 Hz)
2	6.08 *dt* (J = 15.73 and 5.47 Hz)	2	6.07 *dt* (J = 15.7 and 5.39 Hz)
3	3.27 *dd* (J = 5.44 and 1.53 Hz)	3	3.29 *dd* (J = 5.4 and 1.5 Hz)
3′, 5′	6.80 *d* (J = 8.80 Hz)	2′, 5′, 6′	6.81–6.95 *m*
2′, 6′	7.27 *d* (J = 8.80 Hz)	2″, 5″, 6″	
2″, 5″, 6″	6.78–6.98 *m*		

From homonuclear [illegible] (COSY) the obvious coupling [illegible] and between H-3 and H-4 were [illegible]. In addition this procedure also exposed a coupling between protons centred at δ7.32 and 6.84 thus confirming the presence of the A_2B_2 system due to protons (2′, 6′) and (3′, 5′). A comparison of the [1]H NMR spectra for 1 and 3 is shown in Table 2.

EXPERIMENTAL

[1]H NMR spectra were recorded at 80 [illegible] MHz in $(CD_3)_2CO$ solution unless otherwise specified using TMS as internal standard. [13]C NMR spectra were at 20 MHz or 125 MHz.

Isolation of compound **1**. Initial purification of the ethanolic extract of rhizomes from *Hypoxis rooperi* by HPLC using $MeOH-H_2O$ (1 : 1) resulted in an enriched fraction of compound **2**. Subsequent purification of a 10 g portion of this fraction by CC (silica gel) with n-BuOH–C_6H_{14}–MeOH (4 : 3 : 1) afforded hypoxoside (3 g) which crystallized (mp 140–150°) from this solvent mixture on standing.

Hypoxoside (200 mg) and cellulase (Sigma Chemical Company, Type II) (100 mg) were mixed and NaOAc (5 ml) (pH 6.8) added. The stirred reaction mixture was maintained at 37° for 2 hr in a sealed flask whereafter it was diluted with an Me_2CO-H_2O mixture (2 ml) and transferred to a freeze-drying flask. After freeze-drying the [illegible] residue was extracted with Me_2CO and the extract [illegible] at 20° to afford an orange-yellow oil (40 mg). TLC in $C_6H_6-Me_2CO$ (7 : 3) showed the oil to consist of rooperol (3) (R_f [illegible]) and two other components (R_f 0.36 and [illegible]).

Crude rooperol (1 g) was again separated by flash CC (as above) with $C_6H_6-Me_2CO$ (8 : 2) as solvent to give pure rooperol (485 mg, mp 150–152°) and compound 1 (28 mg) as a yellowish-brown solid. Examination of 1 by 200 MHz NMR indicated that it still contained trace quantities of the higher R_f contaminant. Even rechromatography did not remove this [illegible] entirely. [1]H NMR (500 MHz) [illegible] for 1 is shown in Table 2 and [13]C NMR spectra (125 MHz) in Table 1. Methylation with CH_2N_2 gave a brown solid. HRMS m/z 308.1405 [M]$^+$ (calc. for $C_{20}H_{20}O_3$: 308.1412), 293.1177 [$C_{19}H_{17}O_3$] [M-Me]$^+$, 166 (18), 149 (100), 135 (17), 121 (15), 105 (10).

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