Phytochemistry, Vol. 23, No 6, pp. 1313–1316, 1984.
Printed in Great Britain

0031-9422/84 $3.00 + 0.00

ISOLATION OF HYPOXOSIDE FROM *HYPOXIS ROOPERI* AND SYNTHESIS OF (*E*)-1,5-*BIS*(3′,4′-DIMETHOXYPHENYL)PENT-4-EN-1-YNE

SIEGFRIED E. DREWES, ALIDA J. HALL, ROBIN A. LEARMONTH and URSULA J. UPFOLD

Department of Chemistry, University of Natal, Pietermaritzburg, South Africa

(*Revised received* 29 *September* 1983)

Key Word Index—*Hypoxis rooperi*; Hypoxidaceae; corms; hypoxoside; 1,5-diphenylpent-4-en-1-yne; rooperol; synthesis.

Abstract—Isolation of the known diglucoside of (*E*)-1,5-*bis*(3′,4′-dihydroxyphenyl)pent-4-en-1-yne (hypoxoside) from *Hypoxis rooperi* is described. *In vivo* tests indicate low toxic properties. A general synthesis for the introduction of the pentenyne link between two aromatic rings is described.

INTRODUCTION

Very recently Marini-Bettolo *et al.* [1] have described the isolation and characterisation of a new glycoside, named hypoxoside, from the corms of *Hypoxis obtusa*. A review on the closely-related *H. rooperi*, with emphasis on distribution and healing properties of the plant, and emanating from other researchers in this University, appeared some four years ago [2]. We now wish to report our own findings on the extractives of this plant which is widely-distributed in Southern Africa. This work has been in progress in our laboratories for the past three years. Our results are in full agreement with those of Marini-Bettolo [1] with regard to the structure of the major constituent found in *Hypoxis* species, namely the diglucoside of (*E*)-1,5-*bis*(3′,4′-dihydroxyphenyl)pent-4-en-1-yne (**1**). In this paper we will only detail information which is not already covered by the original paper [1].

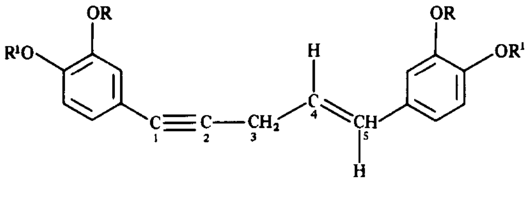
In discussing the properties of extractives from *Hypoxis obtusa* Marini-Bettolo [1] states that infusions of the plant are used by African people for combating urinary diseases. Preliminary tests done locally with **1** indicate no or low toxicity on oral (> 4000 mg/kg) and intraperitoneal (LD_{50} 0 for 500 mg/kg) administration to mice and on intravenous (> 100 mg/kg) administration to rabbits. When given orally (up to 100 mg/kg) the compound exhibited no foetotoxic or teratogenic effects in mice [H. Kündig and L. L. Muir, unpublished results]. The results of these investigations will be published elsewhere.

In the present communication we describe the synthesis of the tetramethoxy derivative of (*E*)-1,5-*bis*(3′,4′-dihydroxyphenyl)pent-4-en-1-yne. This aglycone, readily obtainable from *β*-glucosidase hydrolysis of hypoxoside, and for which we propose the trivial name rooperol, contains certain interesting features which may have a bearing on biological activity. These are the two benzene rings, and the unique pent-4-en-1-yne connecting link possessing a central methylene group which is prochiral and also allylic to both the benzene rings.

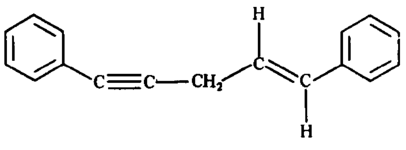
RESULTS AND DISCUSSION

It is surprising that the presence of **1** has escaped attention for so long, since it is present in high concentration in the plant. Marini-Bettolo [1] reports an overall yield from the corms of 3.7 % and we have found the yields to vary between 3.5 and 4.5 %. Hypoxoside is the major constituent of a number of *Hypoxis* species and so far we have obtained it from *H. acuminata*, *H. rooperi*, *H. nitida*, *H. obtusa*, *H. rigidula* and *H. latifolia* and identified it is *Spiloxene schlechteri* (Hypoxidaceae). There is a seasonal variation in concentration of the diglucoside and maximum concentrations remain to be established.

In order to determine the position of attachment of the two glucose units Marini-Bettolo [1] made use of the selective deuteration method of Kirby and Ogunkoya [3]. By this procedure deuteration *ortho* and *para* to a phenolic group occurs. In the case in question, deuteration of a suitable derivative made it possible to establish from the ^1H NMR spectrum that the glucose units resided at the C-4-positions of the benzene rings. We have confirmed this finding by an independent procedure. It involves comparison of the ^{13}C NMR spectrum of 3-methoxy-4-hydroxypropylbenzene (derived from hydrogenation of eugenol) with 1,5-*bis*(3′-methoxy-4′-hydroxyphenyl)pentane (derived from hydrogenation and subsequent methy-



1 R = H, R¹ = glucose



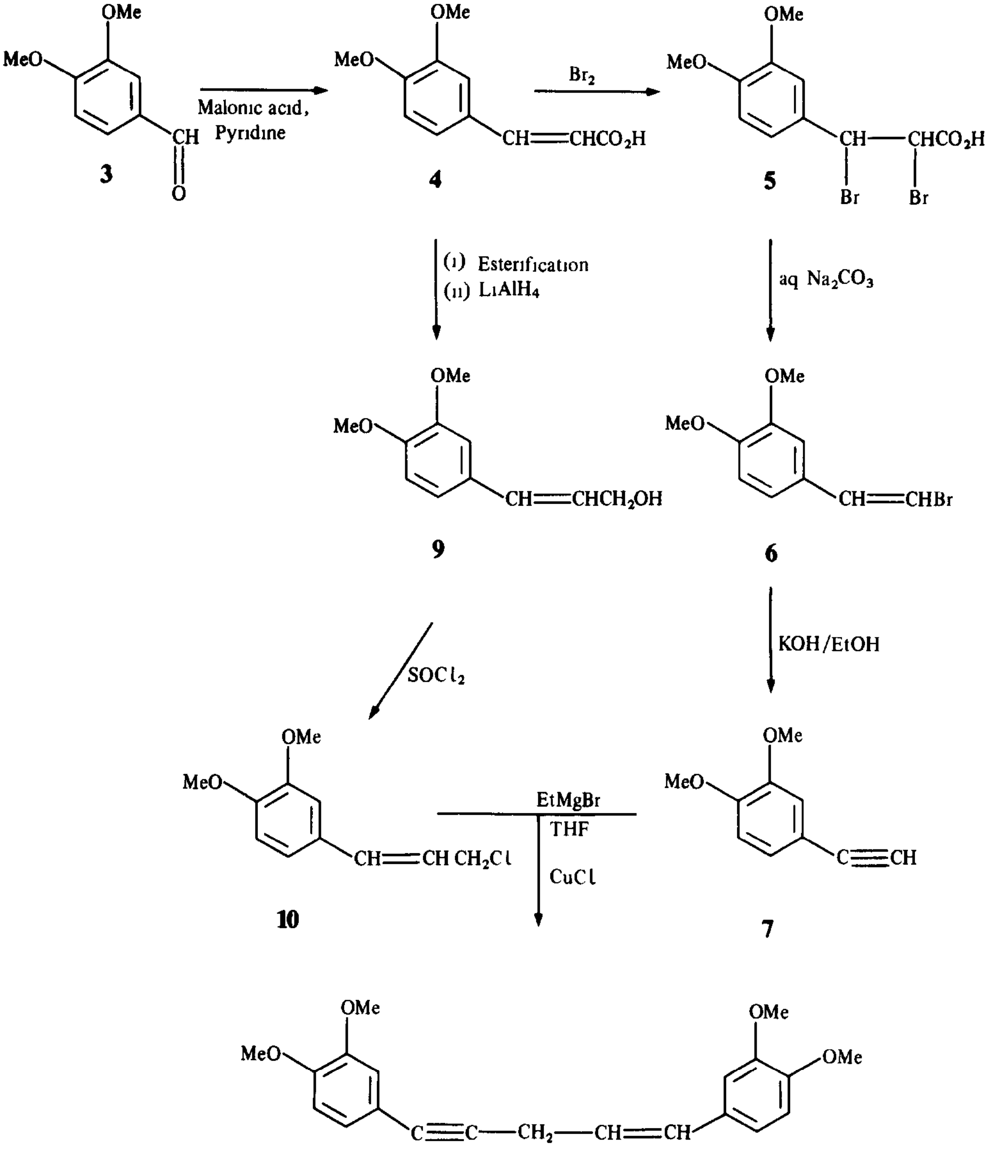
2

Table 1. ^{13}C NMR comparison of aromatic carbons of 3-methoxy-4-hydroxypropylbenzene (**12**) and 1,5-*bis*(3′-methoxy-4′-hydroxyphenyl)pentane (**13**)

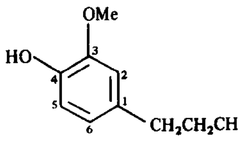
Carbon	Chemical shift [δ-values (ppm)] 12	 13
1	134.26	134.57
2	111.77	112.40
3	147.16	147.73
4	144.40	144.94
5	114.81	115.23
6	120.96	121.15
Benzylic carbon	37.72	35.76
OMe	55.54	55.87

lation and hydrolysis of **1**). The ^{13}C NMR shifts for the aromatic carbons of these two compounds (Table 1) were virtually identical.

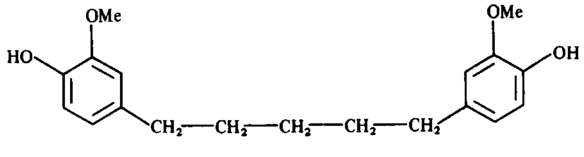
In devising a synthesis of **2** and its analogues we were concerned, in the first instance, to obtain a general procedure for linking two benzene rings (with or without substituents) *via* the novel pentenyne system. Very helpful in this regard was the work of Brandsma on alkynes [4] and the paper by Klein and Brenner [5] on metalation of 1,4-enynes. Our general synthesis, shown for the tetramethoxy derivative (**11**), is outlined in Scheme 1. The synthesis was tested for the simplest case where there are no substituents on the benzene rings [5], to cases where one or both rings bore methoxy, dimethoxy or methylenedioxy substitutents. Two steps in the synthesis were found to give unsatisfactory yields. These were



Scheme 1. Synthesis of 1,5-*bis*(3′,4′-dimethoxyphenyl)pent-4-en-1-yne.



12



13

(i) conversion of the bromoalkene to the alkyne using strong base and (ii) the final 'coupling' of the two units to give the enyne system. Improved conversion for the latter step was achieved by employing the allylic bromo-derivative, rather than the corresponding chloro derivative for the 'coupling' step.

The model synthesis outlined in the Scheme employs vanillin as common starting material for both halves of the target molecule. The end product was identical in all respects to the tetramethyl ether obtained by hydrolysis (β-glucosidase) of glucoside (**1**), followed by methylation with diazomethane.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded at 60 and 80 MHz using TMS as int standard EIMS were by direct insertion into the ionisation chamber. Where accurate masses were determined, a high resolution instrument was used

Isolation of active component (**1**). Freshly pulped corms of *H rooperi* (10 kg) were extracted with EtOH (25 l.) at room temp for 48 hr. Filtration, and concn *in vacuo*, followed by freeze-drying gave a brown powder (729 g). This crude material (10 g) was separated by prep. HPLC using a reverse phase column (PrepPAK-500/C_{18}) with MeOH-H_2O (1.1) as solvent to provide 6.9 g of an enriched fraction. Final separation took place by separating 10 g portions of this material on a silica gel 60 column (230–400 mesh, 45 mm × 600 mm) with 2-butanol–C_6H_6–H_2O–MeOH (4:3 2:1) as solvent. Typically a 10 g fraction yielded 7.9 g of **1** which crystallized with difficulty (mp 148°) from 2-butanol. (Found: C, 54.67; H, 5.87 Calc for $C_{29}H_{34}O_{14}2H_2O$. C, 54 18, H, 5 96%). All other physical data (IR, UV, ^{1}H NMR, ^{13}C NMR) are in good agreement with lit. [1] values.

Decaacetyl derivative of **1** This derivative was prepared by the usual acetylation technique (pyridine–Ac_2O) and gave white needles, mp 134°, from MeOH Lit. [1] mp 127–129°. (Found· C, 57.37, H, 5.39. Calc. for $C_{49}H_{54}O_{24}$: C, 57.31; H, 5.26%) All other data are the same as recorded in ref. [1].

Hydrolysis of **1** Hydrolysis of **1** (100 mg), in H_2O (6 ml, pH 6 3) with β-glucosidase (100 mg in 4 ml H_2O), at 37°, proved to be satisfactory. Our sample of rooperol had mp 148° (lit. mp 154–156°) but otherwise did not differ from that recorded in ref. [1].

Tetramethoxyrooperol. Methylation with CH_2N_2 gave a yellow oil which darkened on standing and did not give a satisfactory elemental analysis HRMS gave 338.152247; $C_{21}H_{22}O_4$ requires 338.151798.

The properties of other derivatives prepared by us, tetraacetoxyrooperol and 1,5-*bis*(3′,4′-dihydroxyphenyl)pentane, were identical to those previously found [1].

^{13}C NMR comparison of 1,5-bis(3′-methoxy-4′-hydroxyphenyl)pentane with 3-methoxy-4-hydroxypropylbenzene. The pentane derivative was prepared essentially as described [1] except that we found that a cleaner end product was obtained if hydrogenation of **1** preceded its methylation. The derivative of eugenol was obtained from hydrogenation (Pt–H_2, MeOH) of a commercial sample. Comparison spectra were run in deuterioacetone (Table 1)

3-(3′,4′-*Dimethoxyphenyl)prop-2-enoic acid* (**4**). This compound was obtained from 3,4-dimethoxybenzaldehyde (**3**) by standard procedures using malonic acid and a base [7], mp 198–202°. (Found: C, 63.44; H, 5.95. Calc. for $C_{11}H_{12}O_4$. C, 63 45; H, 5.76%.)

3-(3′,4′-*Dimethoxyphenyl)-2,3-dibromopropanoic acid* (**5**). Br_2 (7.6 g) was added dropwise to a suspension of **4** (10 g), in $CHCl_3$ (300 ml) Evapn of solvent *in vacuo* provided an off-white solid in almost quantitative yield (17.6 g). ^{1}H NMR (80 MHz, CD_3COCD_3): δ 3.85, 3.89 (2 × 3H, *s*, 2 × OMe) 5.19 (1H, *d*, *J* = 11 8 Hz), 5 50 (1H, *d*, *J* = 11 8 Hz), 6 54 (1H, *s*), 6.95 (1H, *d*, *J* = 8.2 Hz), 7.19 (2H, *m*). No satisfactory C, H analysis could be obtained for this compound.

1-*Bromo*-2-(3′,4′-*dimethoxyphenyl)ethene* (**6**). A 10% aq. Na_2CO_3 soln was added to **5** (14.5 g). After a 12 hr reflux period, the reaction mixture was cooled and extracted with EtOAc. Concn of the extract *in vacuo* gave a residual oil (6.1 g, 64%) which was converted to the alkyne (**7**) without further purification on account of its instability. The ^{1}H NMR spectrum (80 MHz, $CDCl_3$) indicated that it was reasonably pure: δ 3.83 (6H, *s*), 6.57 (1H, *d*, *J* = 14 Hz), 6.78 (3H, *s*) 7.00 (1H, *d*, *J* = 14 Hz). It had intense $[M]^+$ peaks at *m/z* 242 and 244.

3,4-*Dimethoxyethynylbenzene* (**7**). Compound **6** (2.3 g) was finely ground with KOH (1 g) before EtOH (1 ml) was added. The mixture was heated at 130° for 2 hr. After cooling, the product was poured onto ice and extracted with EtOAc. Evapn of solvent gave a brown residue (0.62 g, 40%) which was crystallized from Me_2CO, mp 72–73°. MS *m/z* (rel. int.). 162 ($[M]^+$, 100%), 147 (38), 119 (26) 91 (49); ^{1}H NMR (80 MHz, CD_3COCD_3)· δ 3.5 (1H, *s*), 3.84 (6H, *s*) 7.06 (3H, *m*). (Found: 162 067876; $C_{10}H_{10}O_2$ requires 162.068074.)

Ethyl 3,4-*dimethoxycinnamate* (**8**). Compound **4** was esterified with EtOH to give the ester, mp 55–56° (lit. [7], mp 59°) in 82% overall yield. Its purity was proved by elemental analysis and spectroscopic examination.

3,4-*Dimethoxycinnamyl alcohol* (**9**). A soln of **8** (2.8 g) in dry Et_2O (30 ml) at −5° was added dropwise over 45 min (N_2 atmosphere) to a suspension of $LiAlH_4$ (0.8 g) in Et_2O (75 ml) also at −5° The mixture was stirred at 0° for 4 hr when H_2O was added and, after acidification, it was extracted with Et_2O. Removal of solvent afforded a yellow oil which crystallized slowly (1.89 g, 81%), mp 73–74°, (lit. [8] mp 72°). MS *m/z* (rel. int.): 194 ($[M]^+$, 100%), 165 (2), 163 (10), 152 (10), 138 (6). ^{1}H NMR (80 MHz $CDCl_3$): δ 2.84 (1H, *s*), 3.83 (6H, *s*), 4.25 (2H, *d*) 6.07 and 6.27 (each 1H, *dt*, *J* = 16 Hz and *J* = 5 Hz) (Found: C, 67.89; H, 7.10. Calc for $C_{11}H_{14}O_3$: C, 68.04, H, 7.22%)

3,4-*Dimethoxycinnamyl chloride* (**10**). To a soln of **9** (370 mg) in dry CH_2Cl_2 (6 ml), a mixture of pyridine (210 mg) and $SOCl_2$ (270 mg) in CH_2Cl_2 (3 ml) was added at 0° over a period of

15 min. The reaction mixture was refluxed for 1 hr, cooled, washed with H_2O, and concd *in vacuo* at room temp to provide a highly unstable compound (283 mg, 72%). This compound was used immediately for the next step. ^{1}H NMR (80 MHz, $CDCl_3$): δ 3.86 (3H, *s*), 4.22 (2H, *d*) 6.05 and 6.25 (each 1H, *dt*, J = 16 Hz), 6.59 (1H, *d*, J = 16 Hz), 6.91 (3H, *m*).

1,5-bis(3',4'-*Dimethoxyphenyl)pent*-4-*en*-1-*yne* (**11**). EtBr (130 mg) was added to a stirred mixture of Mg turnings (29 2 mg) in dry THF (2 ml) under N_2. After 15 min the reaction was cooled to 25° and the alkyne (**6**, 161 mg) in THF added dropwise. After complete evolution of C_2H_4 the soln was refluxed for 30 min. CuCl (10 mg) was added to the cooled mixture (25°) followed by the dropwise addition of the allylic chloride (**10**, 210 mg) in THF (3 ml). The mixture was refluxed for 30 min, then stirred at room temp for 2 hr NH_4Cl (1.8 g) and KCN (0 2 g) in H_2O (100 ml) was added to quench the reaction. Extraction with Et_2O gave an oil which was chromatographed on a silica gel column using hexane–C_6H_6–Me_2CO (5.4:1) as eluant to yield **11** as a pale yellow oil (77 mg, 23%). MS *m/z* (rel. int.)· 338 ($[M]^+$, 100%) 323 (17), 307 (17). ^{1}H NMR (80 MHz, $CDCl_3$): δ 3.32 (2H, *d*), 3.88 (3H, *s*), 5.99 and 6.19 (each 1H, *dt*, J = 15 Hz and J = 5.6 Hz), 6.63 (1H, *d*, J = 15 Hz), 6 94 (6H, *m*). ^{13}C NMR (20 MHz, $CDCl_3$); δ 23.05, 55 97, 82 68, 85.42, 109.09, 111.18, 111.35, 114.65, 116.06, 119 40, 122.65, 124.85, 130.40, 131.13, 148.78, 149.20. (Found. 338 151917; $C_{21}H_{22}O_4$ requires. 338.151798)

Acknowledgments—The authors thank Essential Sterolin Products (Pty) Ltd for its financial support and the University of Natal for laboratory facilities.

REREFENCES

1. Marini-Bettolo, G. B., Patamia, M., Nicoletti, M , Galeffi, C and Messana, I. (1982) *Tetrahedron* **38**, 1683
2 Walford, S. N. (1979) *Deut. Apotheker* **31**, 642.
3 Kirby, G W. and Ogunkoya, L (1965) *J. Chem. Soc* 6914
4. Brandsma, L (1971) in *Preparative Acetylenic Chemistry*, p 30. Elsevier, Amsterdam.
5 Klein, J. and Brenner, S. (1970) *Tetrahedron* **26**, 5807.
6. Horning, E C. and Koo, J (1951) *J. Am. Chem. Soc* **73**, 5826
7. Perkin, W H and Schiess, E (1904) *J. Chem. Soc* 159
8 Block, E. and Stevenson, R. (1971) *J Org Chem.* **36**, 3453.