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Pyrano-isoflavones with erectile-dysfunction activity from *Eriosema kraussianum*

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Abstract

Five pyrano-isoflavones have been isolated from the rootstock of *Eriosema kraussianum* N. E. Br (Papilionaceae). Spectral data and single crystal X-ray analyses were used for structural elucidation. The most active of the compounds had an activity of 75% of that found in Viagra in the erectile dysfunction test on rabbit penile smooth muscle. © 2002 Published by Elsevier Science Ltd.

Keywords: *Eriosema kraussianum*; Papilionaceae; Pyrano-isoflavones; Biologically active; Erectile dysfunction

1. Introduction

The genus *Eriosema* is one genus of a collection of plants which go under the Zulu indigenous name of ''uBangalala'' in South Africa. Most of the plant species listed under this name are used for the purpose of curing or alleviating impotency (Bryant, 1983; Hutchings, 1996). Our investigations on the genus *Eriosema* form part of a larger programme investigating the constituents and uses of other indigenous plants which fall under the umbrella name ''uBangalala''. It forms part of a National Programme to research and document indigenous knowledge systems (IKS) in South Africa. Reference to other plants in this category is made, for example, in a very recent publication dealing with the commercial market for medicinal plants in the Witwatersrand area of South Africa (Williams et al., 2000).

Eriosema kraussianum is a small shrublet found in grasslands rarely attaining a height of greater than 15 cm. It flowers from October to February. The plant has an extensive and well developed root system and it is this part of the plant that is used for traditional purposes. No chemical work has been done on the plant. If the root of the plant is damaged during collection a dark red sap forms at the point of injury. This may relate to the type of compound isolated from the plant (see later).

2. Results and discussion

From a CH_2Cl_2–EtOH (1:1) extraction of the milled roots five new pyrano-isoflavones (Ingham, 1983) were isolated. They form a closely-related, and partially interconvertible, group of compounds. We have assigned the trivial names kraussianone **1**, **2**, **3**, **4** and **5** to them. Entry into the series was by way of kraussianone **1** which established the basic isoflavone skeleton. It crystallized as pale yellow needles amenable to X-ray analysis (Fig. 1). The molecular formula of $C_{25}H_{22}O_6$ was established from HRMS (m/z M^+ 418.13993, calculated 418.14164). From ^{1}H NMR, ^{13}C NMR and DEPT analysis it was clear that the compound had 13 quaternary carbons, eight CH's (one of which was far downfield at δ 154.8) and four CH_3 carbons. The ^{1}H NMR spectrum (Table 1) gave the following analysis: two sets of methyl groups at δ 1.42 and δ 1.47, two sets of coupled CH protons at δ 5.63 and δ 6.70 ($J = 10.06$ Hz), and the other at δ 5.50 and δ 6.27 ($J = 9.60$ Hz), and singlets at δ 6.37, 6.51 and 7.90. In total there are eight

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CH signals. Two phenolic OH signals appeared at δ 8.32 and δ 12.55 (obviously H – bonded). The main features of the ^{13}C spectrum (Table 2) were two high field quartets at δ 76.6 and δ 78.4, five quartets in the region 105–123 ppm, a further five quartets, obviously bonded to oxygen functionalities clustered between 150 and 161 ppm, and a carbonyl group at 181.9 ppm.

From HMQC spectra the remaining correlations could be established. Of particular significance was the chemical shift position of H-2 and its linkage to C-4, H-9 (across oxygen), H-2′, C-3 and C-1′. Other correlations include: H-8 to C-7, C-9, C-10; H-3′ to C-4′, C-2′, C-1′; H-6′ to C-2′, C-4′, C-3, C-4″; H-4‴ to C-7, C-6‴, C-5. The far downfield OH group (at δ H 12.56) is undoubtedly attached to C-5 [see also X-ray confirmation (Fig. 1) of this] and the second OH (δ H 8.32) at C-2′.

NOESY correlations are shown in Fig. 2. The correlations confirm the observation from the X-ray analysis (Fig. 1) that rotation about the C-1′ - C-3 bond allows H-2 and H-6′ to approach one another closely.

Examination of the spectral data for **2** ($C_{25}H_{24}O_6$) suggested a close similarity to **1**, except that *either* ring E *or* ring D was in the open chain form i.e. with a 3,3-dimethylallyl side chain on one of the aromatic rings. The latter side chain is an obvious feature of the ^1H NMR spectrum with resonances at δ 3.39 ($J = 6.8$ Hz), δ 5.27 ($J = 6.8$ Hz), δ 1.68 and δ 1.80 for H-1‴, H-2‴, H-4‴ and H-5‴ (resp.). The proton and ^{13}C spectra are summarized in Tables 1 and 2. The structure shown for **2**, in which the side chain is attached at C-6 of ring A, while ring D is unaltered compared with kraussianone **1**, is based on analysis of the ^1H and ^{13}C spectra, and particularly the correlations which were forthcoming from the HMQC and NOESY spectra. The molecular formula $C_{25}H_{24}O_6$, with two more hydrogens than compound **1**, can be accommodated by a free OH at C-7, and an additional hydrogen in the dimethylallyl side chain. The HMQC spectra show correlations of H-5″ with C-5′, C-6″ and C-7″/8″. H-4″ correlates with C-6′, C-5′ and C-6″. This clearly establishes the relationship of rings C and D with one another. There is a strong correlation of H 4‴/5‴ with C-3‴ and C-2‴ while H-1‴ is linked to C-7, C-5, C-3‴ and C-2‴, thus proving the nature and position of this side chain. The remaining correlations are all in agreement with the proposed

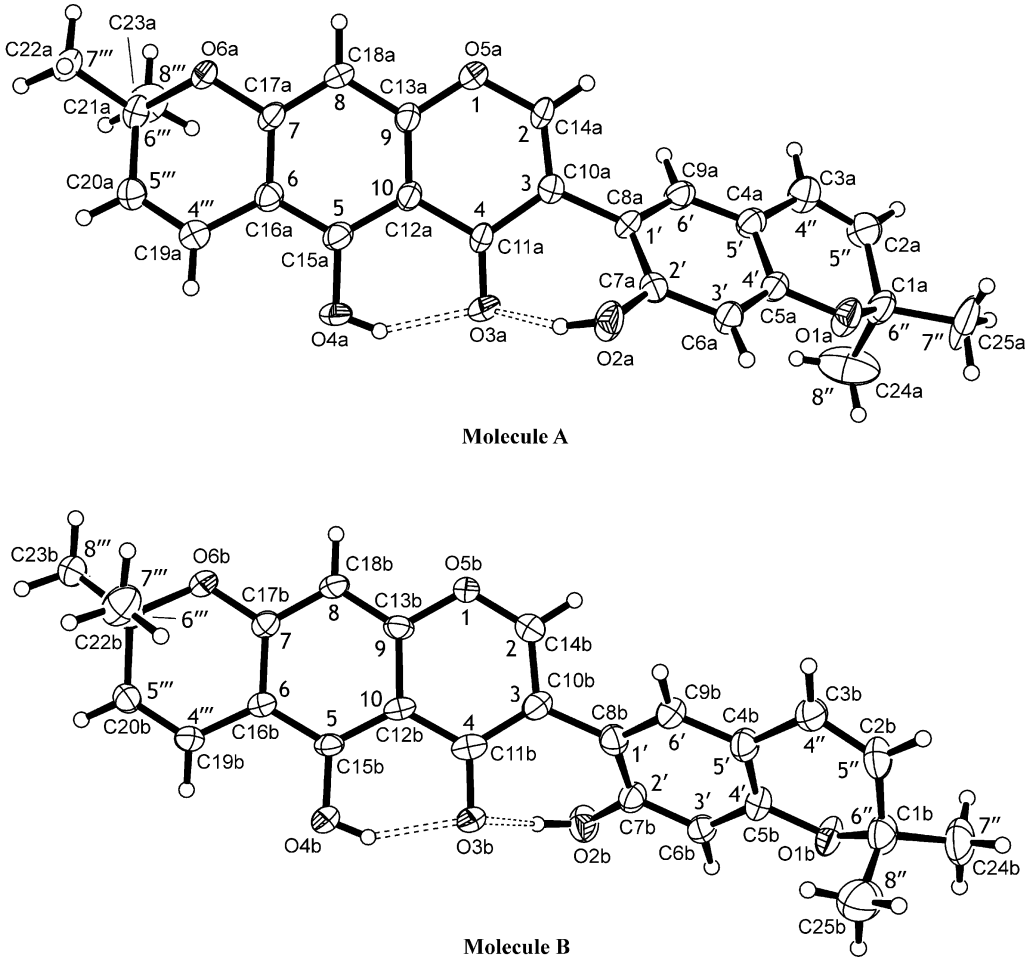

Fig. 1. X-ray structure of kraussianone **1** (40% thermal ellipsoids).

Table 1
^{1}H NMR date for compounds **1**, **2**, **3**, **4** and **5** (500 MHz)

Proton	**1** δH (mult. *J* in Hz)	**2** δH (mult. *J* in Hz)	**3** δH (mult. *J* in Hz)	**4** δH (mult. *J* in Hz)	**4** δH (mult. J in Hz)	**5** δH (mult. *J* in Hz)
2	7.90(*s*)	7.96(*s*)	8.15(*s*)	6.23(*s*)	6.22(*s*)	6.12(*s*)
3	–	–	–	–	–	–
8	6.37(*s*)	6.62(*s*)	6.44(*s*)	5.93(*s*)	5.94(*s*)	5.97(*s*)
3′	6.51(*s*)	6.48(*s*)	6.30(*s*)	6.37(*s*)	6.42(*s*)	6.30(*s*)
6′	6.73(*s*)	6.80(*s*)	6.87(*s*)	7.03(*s*)	7.05(*s*)	6.91(*s*)
4″	6.26(*d*, 9.6)	6.27(*d*, 10.0)	6.29(*d*, 9.6)	6.25(*d*, 10.0)	6.34(*d*. 9.6)	6.13(*d*, 10.1)
5″	5.50(*d*, 9.6)	5.51(*d*, 10.0)	5.52(*d*, 9.6)	5.52(*d*, 10.1)	5.61(*d*, 9.6)	5.40(*d*, 10.1)
4‴	6.70(*d*, 10.1)	1.68(*s*)	1.14(*s*)	6.55(*d*, 10.1)	6.54(*d*, 9.8)	1.57(*s*)
5‴	5.63(*d*, 10.1)	1.80(*s*)	1.14(*s*)	5.53(*d*, 10.0)	5.63(*d*, 9.8)	1.66(*s*)
7″ Me	1.42(*s*)	1.42(*s*)	1.35(*s*)	1.36(*s*)	1.36(*s*)	1.27(*s*)
8″ Me	1.42(*s*)	1.42(*s*)	1.35(*s*)	1.40(*s*)	1.40(*s*)	1.31(*s*)
7‴ Me	1.47(*s*)	–	–	1.40(*s*)	1.38(*s*)	–
8‴ Me	1,47(*s*)	–	–	1.44(*s*)	1.42(*s*)	–
2′ OH	8.32(*s*)	9.13(bs)	10.72(b*s*)	–	–	–
5 OH	12.55(*s*)	12.60(*s*)	13.15(*s*)	12.05(*s*)	12.05(*s*)	11.74(*s*)
7.OH	–	8.51(*bs*)	9.56(*s*)	–	–	8.90(*s*)
1‴	–	3.39(*d*, 6.8)	2.58(*t*, 8.2)			3.16(*d*, 7.3)
2‴	–	5.27(*d*, 6.8)	1.51(*t*, 8.2)			5.09(*dd*, 7.3)
Solvent	($CDCl_3$/$(CD_3)_2CO$)	DMSO-d_6	($CDCl_3$/$(CD_3)_2CO$)	($CDCl_3$/$(CD_3)_2CO$)	CD_3CN	($CDCl_3$/$(CD_3)_2CO$)

Table 2
^{13}C NMR data for compounds **1**, **2**, **3**, **4** and **5** (125 MHz in $CDCl_2$)

Proton	**1** δC (mult.)	**2** δC (mult.)	**3** δC (mult.)	**4** δC (mult.)		**5** δC (mult.)
2	154.8(CH)	154.7(CH)	155.4(CH)	110.5(CH)	111.2(CH)	109.6(CH)
3	122.9(C)	122.2(C)	120.0(C)	78.0(C)	78.5(C)	77.7(C)
4	181.9(C)	181.7(C)	180.5(C)	192.2(C)	193.2(C)	191.1(C)
5	156.3(C)	159.2(C)	158.9(C)	158.5(C)	159.3(C)	161.5(C)
6	115.3(C)	112.5(C)	112.9(C)	103.7(C)	104.6(C)	109.5(C)
7	160.3(C)	162.3(C)	162.2(C)	163.1(C)	163.7(C)	164.9(C)
8	94.9(CH)	93.4(CH)	93.0(CH)	96.9(CH)	97.3(CH)	95.6(CH)
9	157.1(C)	155.8(C)	155.4(C)	159.2(C)	159.8(C)	157.3(C)
10	105.4(C)	104.9(C)	104.4(C)	100.2(C)	100.9(C)	99.4(C)
1′	112.0(C)	112.0(C)	110.7(C)	118.5(C)	119.3(C)	118.3(C)
2′	157.1(C)	156.8(C)	156.6(C)	160.4(C)	160.9(C)	160.0(C)
3′	107.2(CH	106.5(CH)	103.4(CH)	99.7(CH)	100.3(CH)	99.6(CH)
4′	155.5(C)	155.2(C)	153.8(C)	156.7(C)	157.4(C)	156.4(C)
5′	106.0(C)	114.9(C)	112.6(C)	116.7(C)	117.7(C)	116.5(C)
6′	127.2(CH)	127.6(CH)	129.5(C)	122.6(CH)	123.5(CH)	121.9(CH)
4″	121.3(CH)	121.4(CH)	121.7(CH)	121.9(CH)	122.3(CH)	121.6(CH)
5″	128.6(CH)	128.3(CH)	127.6(CH)	128.7(CH)	129.9(CH)	128.3(CH)
6″	76.6(C)	76.5(C)	76.3(C	77.2(C)	78.1(C)	76.8(C)
7″	28.1(CH3)	28.0(CH3)	27.9(CH3)	28.1(CH3)	27.3(CH3)	27.7(CH3)
8″	28.1(CH3)	28.0(CH3)	27.9(CH3)	28.1(CH3)	27.4(CH3)	27.8(CH3)
1‴	–	21.4(CH2)	17.4(CH2)	–	–	20.8(CH2)
2‴	–	121.7(CH)	42.5(CH2)	–	–	121.5(CH)
3‴	–	132.2(C)	69.2(C)	–	–	132.1(C)
4‴	115.2(CH)	25.6(CH3)	29.3(CH3)	114.9(CH)	115.1(CH)	25.5 (CH3)
5‴	128.7(CH)	17.7(CH3)	29.3(CH3)	126.9(CH)	128.4(CH)	17.5(CH3)
6‴	78.4(C)	–	–	78.9(C)	79.9(C)	
7‴	28.4(CH3)	–	–	28.5(CH3)	27.7(CH3)	
8‴	28.4(CH3)	–	–	28.5(CH3)	27.8(CH3)	
Solvent	($CDCl_3$/$(CD_3)_2CO$)	($CDCl_3$/$(CD_3)_2CO$)	DMSO-d_6	$(CD_3)_2CO$	CD_3CN	($CDCl_3$/$(CD_3)_2CO$)

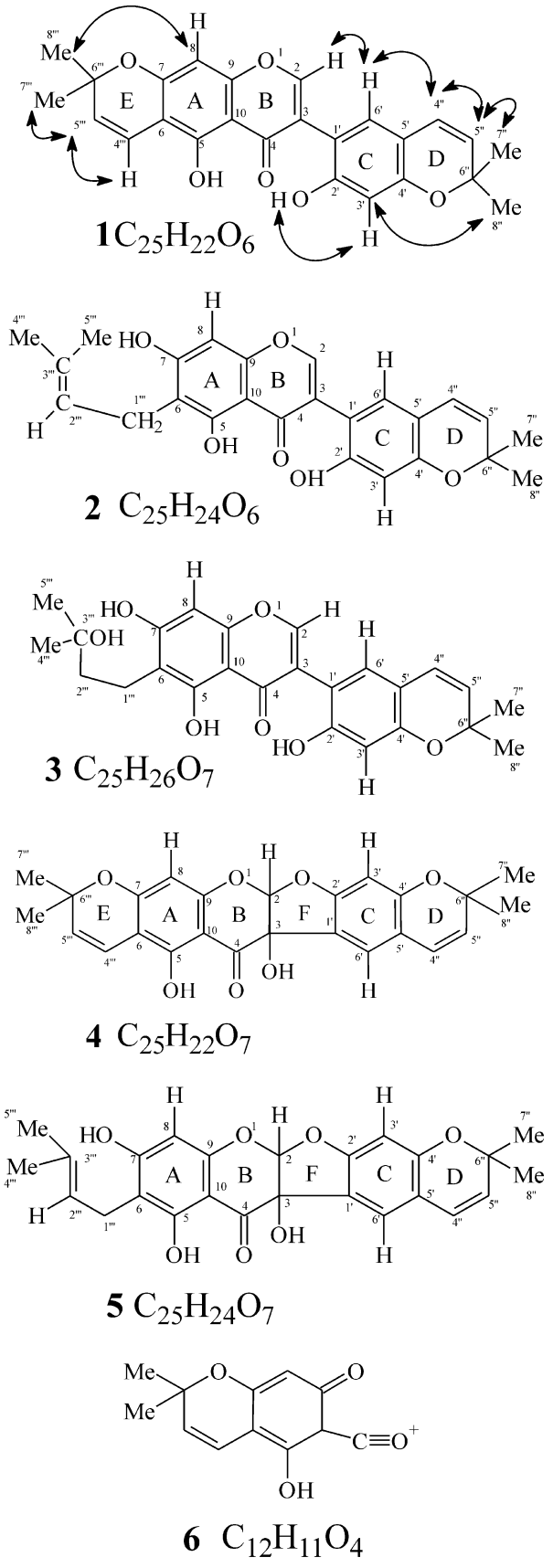

Fig. 2. NOESY correlations for kraussianones **1**–**6**.

structure (Table 3). As was the case in compound **1**, there is a good NOESY correlation between H-2 and H-6′, emphasizing the fact that rotation of the C-3–C-1′ bond allows these two protons to come close together. The NOESY evidence for the close proximity of the proton on C-2‴ to the C-4‴ methyl group on C-3‴ clarifies the stereochemistry around the C-2‴–C-3‴ double bond. Proton and ^{13}C spectra are collected in Tables 1 and 2.

The third compound, kraussianone **3**, $C_{25}H_{26}O_7$, with two more hydrogens than **2**, and an additional oxygen, crystallized readily and was amenable to X-ray analysis. It was considerably more polar than the two foregoing compounds and was light orange in colour. In this instance spectral analysis again indicated an intact D ring, with rings A, B and C similar to those in **1** and **2**. DEPT and HMQC analysis showed unambiguously that a 3, 3-dimethyl-3-hydroxybutyl side chain was attached to C-6 i.e. a hydrated 3,3-dimethylalyl side chain. The relationship of the C-1‴ protons to the surrounding atoms is shown up well in the HMQC spectrum. Positive correlations extend to C-7, C-5, C-6, C-3‴ and C-2‴. This technique also makes it possible to distinguish between the singlet methyl groups arising from the 4‴/5‴ methyls and the 7″/8″ methyls (Tables 1 and 2). The mass spectrum exhibits a strong molecular ion peak at M^+ 438 and the anticipated losses of methyl radical and subsequently water are shown by prominent peaks at m/z 423 and 405 (base peak) respectively.

Kraussianone **4**, while obviously related to **1**, **2** and **3**, also exhibited several differences. Its molecular formula from high resolution mass spectrometry is M^+ 434.13677 ($C_{25}H_{22}O_7$). This is the same as for compound **1**, except for an additional oxygen atom. Mass fragmentation gives rise to a large fragment ion at m/z 419 (loss of methyl radical) and the base peak is at m/z 219. The latter probably represents the ion **6**, a fragment typical of a retro Diels–Alder fission (Drewes, 1974).

In the ^1H NMR spectrum of **4**, H-2, typically resonating at ca. δ 8.0 in **1**, **2** and **3**, is absent. In its place a "new" singlet peak is observed at δ 6.22 assigned to H-2. In the ^{13}C spectrum the peaks normally associated

Table 3
HMQC correlations for kraussianone **2**

Proton	Coupling to
H-2	C-4(C=O), C-9, C-3, C-1′
H-3′	C-2′, C-4′, C-1′
H-6′	C-2′, C-4′, C-4″
H-8	C-4, C-7, C-9, C-10, C-6
H-4″	C-4′, C-6′, C-5′, C-6″
H-5″	C-6″, C-5′
H-1‴	C-7, C-5, C-3‴, C-2‴,C-6
H-4‴	C-3‴, C-2‴
H-5‴	C-3‴, C-2‴
7″, 8″ Me	C-5″, C-4″, C-6″

with C-6″ and C-6‴ (at about δ 77 and δ 79) are still present, but there is an additional peak in the same chemical shift region (δ 78.01) belonging to C-3. Following detailed analysis of DEPT, HSQC, HMQC and NOESY spectra, the structure shown in **4** was proposed. A possible pathway involving ring closure of the phenolic hydroxyl at C-2′ (see structure **1**) at position C-2 (in ring B) by a conjugated mechanism, followed by hydroxylation at C-3 (of ring B) is postulated. Ring closure thus leads to the formation of an additional furan ring F, in the system. The compounds lisetin (Falshaw et al., 1966) and milletin (Raju et al., 1981) have systems of this nature.

Allocation of the ^{1}H and ^{13}C resonances for **4** (run in $CDCl_3/(CD_3)_2CO$) are shown in Tables 1 and 2. The spectral evidence for allocation of all carbons and hydrogens shown in structure **4** is unambiguous. The evidence presented below relates specifically to atoms at C–2 and C-3 (ring F) and C–4 (ring B) since the situation here is unlike that found in compounds **1**, **2** and **3**. It is instructive to compare the proton and ^{13}C chemical shifts of these atoms in **4**, with those in compound **1** (Table 4).

Since H-2 is part of an acetal system in **4** (as opposed to an sp^2 system as in **1**) the observed upfield shift is expected. The carbon shifts of C–2 and C–3 reflect accurately the changed magnetic environment in compound **4**, and the downfield shift of the carbonyl group in **2** is in line with the chemical shift for a less conjugated carbonyl. Cross peaks in the HMQC spectrum link H-2 with C-2′, C-9, C-1′, C-3 and C-4 as can be anticipated. Apart from the obvious NOESY correlations, the only other strong correlation detected was that between H-6′ and H-4″.

Since the proton resonance for H-2 (singlet) in **4** overlapped with the doublet due to H-4″ in $CDCl_3/(CD_3)_2CO$ as solvent, CD_3CN was examined as alternative with excellent results. In this solvent H-2 and H-4″ are well separated (Table 1) and the overlapping doublets of H-5‴ and H-5″ are also separated as two distinct doublets. In addition in CD_3CN there is the additional advantage that C-3 is not "hidden" under overlying chloroform peaks. In this solvent, apart from the cross coupling peaks seen for H-2 (see above), additional long-range couplings could be detected. Thus, C-3 (at δ 78.8) is cross-coupled to H-6′ and the phenolic OH at δ 12.6 cross couples to C-5. This is significant since it demonstrates that there is only one phenolic OH in **4**, and that it resides at C-5.

The final compound in the series is kraussianone **5**[1] chemically closely-related to kraussianone **4**. This was an important find in the plant since it gave us the opportunity to examine the structural detail of a second representative of the "furano" isoflavone series. It differs from the first compound in the series kraussianone **4**, only by having the "open chain" system attached to ring A. The proton NMR of **5** (Fig. 1) shows a striking resemblance to that of **4**. The major differences are the appearance of an additional OH- signal at δ 8.90, a triplet at δ 5.09, a doublet at δ 3.16 and a simpler methyl resonance region. This is indicative of a compound identical to kraussianone **4**, but with a 3, 3-dimethylallyl side chain at C-6 instead of ring E. The molecular formulae was found to be $C_{25}H_{24}O_7$, i.e. two hydrogens more than **4**. The additional H's are due to the free phenolic group at C-7 (explaining the new peak at δ 8.90) and the methylene group in the dimethylallyl side chain. The ^{13}C spectrum is in good accord with the

Table 4
Comparison of chemical shifts (δ) of atoms at positions 2, 3 and 4 in compounds **1** and **4**

^{1}H NMR	Compound	
	1	**4**
H-2	7.90	6.22
^{13}C NMR		
C-2	154.8 (sp^2 carbon)	110.5 (sp^3 carbon)
C-3	122.9 (sp^2 carbon)	78.0 (sp^3 carbon)
C-4	181.9 (C=O, doubly conjugated)	192.2 (C=O, singly conjugated)

Table 5
HMQC correlations for kraussianone **5**

Proton	Coupling to
H-2	C-2′, C-9, C-1′
H-3′	C-2′, C-4′, C-1′, C-5′
H-6′	C-2′, C-4′, C-4″, C-3′, C-3
H-8	C-7, C-9, C-6, C-10
H-1‴	C-7, C-5, C-3‴, C-2‴,C-6
H-2‴	C-5‴, C-4‴,1‴
H-4″	C-4′, C-6′, C-5′, C-3′, C-6″
H-5″	C-5′, C-6″
H-7″ (Me)	C-5″, C-6″
H-8″ (Me)	C-5″, C-6″
H-4‴ (Me)	C-2‴, C-3‴
H-5‴ (Me)	C-2‴, C-3‴

Table 6
Percentage carvenosal smooth muscle relaxation by kraussianones **1**, **2** and Viagra at 78 ng/ml

Replicate	Kraussianone **1**	Kraussianone **2**	Viagra
1	80	75	100
2	75	60	100
3	85	60	100
4	–	67	–
5	100	67	–
Average	85	65	100
s.d.	10.8	6.2	0

[1] Proposed structure subsequently confirmed by X-ray analysis (January 2002).

proposed structure (Table 2). The long-range connections obtained from the HMQC spectrum are very convincing and are depicted in Table 5. The clear connectivity of H-2 with both C-9 and C-2′ as well as C-1′ serves to confirm its assigned position in the structure.

2.1. Biological activity

In order to examine the properties of the kraussianones for the traditional usage as agents to cure impotence (''uBangalala'') a standard procedure (Levin et al., 1997) for examining the smooth muscle relaxation of rabbit penile muscle was employed. This test is based on the veno-occlusive mechanism (Godschalk et al., 1997) which depends on facilitating increased arterial flow into the penis (via relaxation of the relevant smooth muscles) and decreased venous outflow so that pressure within the penis rises and it becomes rigid. In order to compare the activity of the new compounds, sildanafil (Viagra), was used as the reference substance. The test is effectively a procedure which measures male erectile dysfunction.

At a concentration of 78 ng/ml the results shown (Table 6) were obtained (the effect obtained is dose-dependent). To our pleasant surprise kraussianone **1** showed an activity close to that of Viagra, thus living up to the plant's traditional use. At this preliminary stage the mode of action and many other details are unknown and await further investigations. Compounds **3**, **4** and **5** did not show relaxation of smooth muscle.

It is of interest to note that the pyranoisoflavones isolated from gorse (*Ulex eurapaeus*, Russell et al., 1990) were examined for their insect feeding deterrent activity. The prenylated chromones from *Eriosema tuberosum* (Ma et al., 1995, 1996a,b) were all found to be active as antifungal reagents. Ma et al. (1995), however, make the interesting observation that ''Indians around Kunana, Venezuela, use the root decoction of *E. rufum* against sterility in women and give it to accelerate delivery in childbirth''. Our present finding highlighting the activity of *Eriosema kraussianum* in the sexual/reproductive field is thus not without precedent in other parts of the world, and emphasizes the crucial role which accurate indigenous knowledge can play in uncovering new uses of pharmaceutical products.

2.2. X-ray structures of *1* and *2*

The X-ray structure of **1** is depicted in Fig. 1. Four observations can be made:

(i) two molecules are found in each unit cell,
(ii) hydrogen bonds exist between the C-5 hydroxyl group and the C=O on C-4 as well as a similar H- bond between the 2′- hydroxyl group and the C-4 carbonyl,
(iii) the dihedral angle between C-2, C-3, C-1′ and C-6′ is 48.2° (molecule A). When the ring C rotates about the C-3–C-1′ intercarbon bond to the alternative conformation the corresponding dihedral angle is −49.4° (molecule B).

Semi-empirical quantum mechanics calculations (AMI gas phase) indicate that the most stable conformation of the molecule differs only little from that assumed in the solid (crystal) state. It is of interest to note, that, in keeping with the above observations a strong NOE effect is seen between H-2 and the hydrogen attached to C-6′, thus confirming the close proximity of these two atoms.

The X-ray structure of **3** is shown in Fig. 3. Again there are two molecules in the unit cell, and the conformations are identical. More advanced molecular simulations for both **1** and **3** are underway and these results will be published elsewhere.

3. Experimental

3.1. General

^{1}H and ^{13}C spectra were recorded on a Varian 500 spectrometer. High resolution mass spectra were measured on a Kratos MS 80 RF double-focussing magnetic sector instrument at 70 eV. X-ray studies were carried out on a Nonius CAD 4 diffractometer with graphite monochromated MoK_α radiation.

3.2. Plant material

Eriosema kraussianum N. E. Br was collected in October 1999 from open veld adjacent to the National Botanical Gardens in Pietermaritzburg. Flowering material was identified by Dr. T. Edwards, curator of the Bews Herbarium at the University of Natal, Pietermaritzburg. A voucher specimen (S. E. D. No. 7) of the whole plant was deposited in the Herbarium.

3.3. Extraction and isolation

Plant material (rootstock, 670 g) from *Eriosema kraussianum* was finely milled and extracted with CH_2Cl_2 for 9 days to give a brown powder (4.1 g). Subsequent extraction with CH_2Cl_2/EtOH (50:50) afforded a further 5.6 g of material.

The CH_2Cl_2 extract showed (on a TLC plate run in CH_2Cl_2 as solvent) five UV fluorescent bands (staining various shades of blue with acid/anisaldehyde reagent) at R_f values of 0.65, 0.39, 0.17, 0.08 and 0.00. These spots were subsequently identified (see below) as

kraussianones **1**, **4**, **2**, **5** and the more polar kraussianone **3**, respectively.

Separation of the crude CH_2Cl_2 extract (3 g) on a silica gel column (Merck 09835) using $CHCl_3/CH_2Cl_2$ (1:1) gave several fractions. From the first and second fractions two compounds were isolated, **1** (60 mg) and **4** (4.7 mg). The latter was obtained in pure form only after additional separation on a silica gel column eluted with EtOAc/petroleum ether bp 40–60 °C (1:1).

In order to obtain the next compound **2** the CH_2Cl_2/EtOH (1:1) extract (see above) was utilized. The extract (5.65 g) was first partitioned between water and EtOAc, and the organic phase (3 g) then fractionated on a silica gel column using gradient elution with hexane/EtOAc. The first fractions provided a mixture of **1** and **4** (220 mg) and subsequently the major compound **2** (700 mg crude product, giving 220 mg of fairly pure material) was eluted. High purity was only achieved after several additional separations on a chromatotron with CH_2Cl_2 as solvent.

For the last two compounds, **3** and **5**, a more polar elution system was employed. Crude CH_2Cl_2 extract (3.7 g) was fractionated using a succession of solvents: CH_2Cl_2, then CH_2Cl_2/MeOH (94:6) and finally Et_2O. The mustard-yellow ether fractions were pooled, concentrated to a small volume and hexane added. Orange crystals of **3** (40 mg) formed slowly and were subjected to X-ray analysis.

To obtain **5** the following procedure gave the best yield: CH_2Cl_2 extract (2.87 g) was fractionated successively with $CHCl_3$, CH_2Cl_2 and Et_2O (dry-packed silica gel column). A portion of the ether eluent was purified on a chromatotron (hexane/Et_2O gradient) and afforded more **3** (90 mg). The remainder of the ether solution (1.65 g) was fractionated on the chromatotron CH_2Cl_2/ether (80:20) to yield **5** (30 mg) as an oil.

*3.4. Kraussianone (**1**). 5,2′-Dihydroxy-[(6″,″-dimethylpyrano (2″,3″:4′,5′)][(6‴,6‴-dimethylpyrano (2‴,3‴:7,6)]-isoflavone*

Yellow crystals mp 185–187 °C, no optical rotation. ^1H and ^{13}C spectral data (500 and 125 MHz resp.,

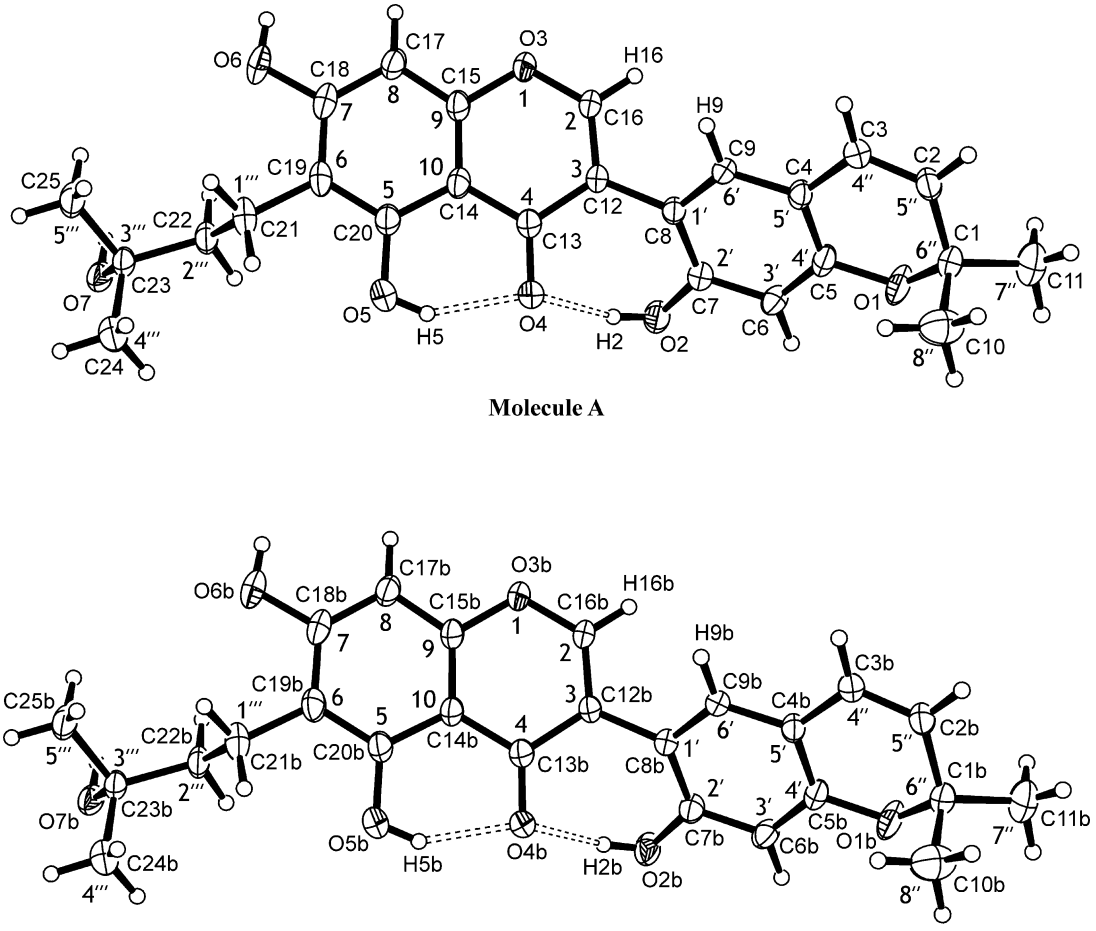

Fig. 3. X-ray structure of kraussianone **3** (40% thermal ellipsoids).

$CDCl_3/(CD_3)_2CO$) in Tables 1 and 2, respectively; H–R EI–MS *m/z* 418.13993 M^+, calcd. for $C_{25}H_{22}O_6$, 418.14164, EI–MS *m/z* (rel. int.): 418[M^+] (25), 403(100), 203(15), 194(21), 185(10); IRν_{max} (KBr) cm^{-1}: 3035, 1650, 1541 and 1132. The X-ray structure is shown in Fig. 1.

3.5. Kraussianone (2). 5,7,2′-Trihydroxy-6-(3,3-dimethylallyl)-[(6″,6″-dimethylpyrano(2″, 3″:4′,5′)]-isoflavone

White crystals, mp 162–168 °C, no optical rotation. ^1H and ^{13}C NMR (500 and 125 MHz resp., $CDCl_3/(CD_3)_2CO$ spectra data in Tables 1 and 2, respectively; H–R EI–MS *m/z* 420.15703 M^+ calc. for $C_{25}H_{24}O_6$, 420.15729, EI–MS *m/z* (rel. int.): 420 [M^+] (28), 405(100), 377(6), 349(23), 201(18), 165(15).

3.6. Kraussianone (3). 5,7,2′-Trihydroxy-6-(3-hydroxy-3-methylbutyl)-[(6″,6″-dimethylpyrano (2″,3″:4′,5′)]-isoflavone

Orange yellow crystals, softening 156 °C melting 218–220 °C, no optical rotation. ^1H and ^{13}C NMR spectra (500 and 125 MHz resp., DMSO-d_6) are in Tables 1 and 2, respectively; H–R EI–MS *m/z* 438.16801 M^+ calc. for $C_{25}H_{26}O_7 = 438.16785$, EI–MS *m/z* (rel. int.): 438 [M^+] (54), 423(58), 405(100), 349(83), 185(36), 175(23), 165(15). The X-ray structure is shown in Fig. 3.

3.7. Kraussianone (4). 5b,7-Dihydroxy-2,2,10,10-tetramethyl-5b,13a-dihydro-2H,6H,10H-chromeno [6′,7′:4,5]furo[2,3-b]pyrano[3,2-g]chromene-6-one

In the text this compound as well as kraussianone **5**, retain the numbering used for the other pyrano-isoflavones in order to simplify cross-referencing.

Pale yellow oil, no optical rotation. ^1H and ^{13}C NMR spectra (500 and 125 MHz resp., $(CDCl_3/(CD_3)_2CO)$ and CD_3CN) are shown in Tables 1 and 2, respectively; H–R EI–MS *m/z* 434.13677 M^+ calc. for $C_{25}H_{22}O_7 = 434.13655$, EI–MS *m/z* (rel. int.): 434 [M^+] (38), 419(84), 219($C_{12}H_{11}O_4$)(100), 201(60), 187(13).

3.8. Kraussianone (5). 5b,7,9-Trihydroxy-2,2-dimethyl-8-(3-methyl-2-butenyl)-5b,11a-dihydro-2H,6H-chromeno[6′,7′:4,5]furo[2,3b]chromen-6-one

Oil, no optical rotation. ^1H and ^{13}C NMR spectra (500 and 125 MHz resp., $(CDCl_3/(CD_3)_2CO)$ are shown in Tables 1 and 2 respectively; H–R EI–MS *m/z* 436.15272 M^+ calc. for $C_{25}H_{24}O_7 = 436.15220$, EI–MS *m/z* (rel.int.): 436 [M^+] (68), 421(94), 403(8), 221($C_{12}H_{13}O_4$)(97), 201(100), 187(16), 183(14), 165(62).

3.9. X-ray data

Details regarding the crystal structure of compounds **1** and **3** are collected in Table 7. See also Figs. 1 and 3.

3.10. Measurement of rabbit corpus cavernosum relaxation

The bioassay was done as described (Levin et al., 1997) with some minor changes. Strips (12 mm long and 1–2 mm thick) of rabbit corpus carvernosal smooth muscle were dissected and mounted in an organ-bath chamber containing Krebs–PSS solution with the following composition: NaCl = 7.01 g/l, KCl = 0.34 g/l, KH_2PO_4 = 0.1 g/l, $NaHCO_3$ = 1.99 g/l, $CaCl_2$ = 0.2 g/l, $MgSO_4$ = 0.3 g/l and glucose = 1.8 g/I. One end of the muscle was secured to the inside case of the perfusion bath and the other end to the thin wire connected to a Harvard isotonic force transducer for isotonic tension measurements. Changes in isotonic tension were recorded on a computerised calibration program. The corpus cavernosum muscle was perfused with 2 ml Krebs–PSS buffered saline and oxygenated with 95% O_2 and 5% CO_2 for 5 min to ascertain a stable baseline recording. This was followed by perfusion with 2 ml of $CaCl_2$ in Krebs–PSS (17.8 mg/ml) for muscle contraction. Baseline tension was set at the point of maximal contraction following the addition of $CaCl_2$ into the experimental

Table 7
X-ray data for compounds **1** and **3** (Figs. 1 and 3)

Molecular formula	Compound **1** $C_{25}H_{22}O_6$	Compound **3** $C_{25}H_{26}O_7$
Crystals (space group)	Triclinic P1	Triclinic P1-bar
Unit cell dimensions (A°)	$a = 8.0583(10)$	$a = 12.250(7)$
	$b = 8.7319(8)$	$b = 12.250(9)$
	$c = 15.4795(14)$	$c = 17.672(9)$
Z	1	2
Volume ($A^{\circ 3}$)	1023.34(18)	2130.0(19)
Crystal size (mm)	0.70 × 0.60 × 0.40	0.55 × 0.35 × 0.30
Unique reflections collected	4471/4336 [R(int) = 0.0029]	8377/7498 [R(int) = 0.0245]
Final R indices [I > 2 sigma (I)]	$R1 = 0.0356$, wR2 = 0.1038	$R1 = 0.0729$, $wR2 = 0.2138$
R indices (all data)	$R1 = 0.0399$, wR2 = 0.1106	$R1 = 0.0940$, $wR2 = 0.2275$

bath. The compounds to be analysed were added after a stable contraction baseline had been obtained. The final compound concentration in the perfusion bath was 78 ng/ml. The same procedure (and concentration) was repeated for the positive control, Viagra. In these experiments the stimulation frequency used for rabbit strips was 9 Hz.

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