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Minor pyrano-isoflavones from *Eriosema kraussianum*: activity-, structure-, and chemical reaction studies

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Abstract

The isolation and identification of two minor pyrano-isoflavones from *Eriosema kraussianum* is described. New studies on the original pyrano-isoflavones shows that: (i) kraussianone **2** (a major compound in the plant) can be cyclised under acid conditions, (ii) kraussianones **3** and **5** cause contraction (not relaxation as anticipated) of *corpus cavernosum* tissue and (iii) the structures proposed previously for **4** and **5** are confirmed by the data obtained from an X-ray study of **5**.

Keywords: *Eriosema kraussianum*; Pyrano-isoflavones; Cyclization; Smooth muscle contraction; X-ray

1. Introduction

In our earlier communication (Drewes et al., 2002) we reported on the isolation and erectile-dysfunction (E-D) activity of five pyrano-isoflavones (**1**–**5**) from *Eriosema kraussianum* (Papilionaceae). From the start of our work it was clear from TLC studies on the crude extract that several very minor components accompanied the above "major" five, and that these needed to be isolated. This has now been achieved.

2. Results and discussion

Compound **6** provided no major structural surprise since it closely resembles **1**. Its dehydration at C-4‴–C-5‴ should convert it to **1**. Compound **7** presented a variation in the sense that in compounds **1**–**6** there is always a geminal dimethyl group in ring E (or its open chain equivalent) whereas in **7** a 2-hydroxy-3-methyl-3-butenyl moiety is linked to ring A.

Repeated fractionation by column chromatography followed by several separations on the chromatotron afforded milligram quantities of **6** and **7**.

In the proton spectrum of **6** the benzylic CH_2 group stands out as a double doublet of doublets (centred at δ2.78 and δ2.99). This appearance was in keeping with the presence of two diastereotopic protons (probably part of a ring structure) coupled with a further proton on an adjacent carbon. Closer examination (^{13}C, COSY, DEPT, HSQC, HMQC spectra) proved the existence of ring E, and confirmed the existence of a CHOH group at C-5‴ coupled to the methylene protons at C-4‴. The resonance positions of the remaining protons and carbons (Tables 1 and 2) bear a close similarity to those found in compounds **2** and **3** and could be assigned readily. Data from the HMQC spectra fully substantiate the nature of ring E. High resolution mass spectrometry confirmed the molecular formula of $C_{25}H_{24}O_7$.

Kraussianone **7** is structurally closely related to **6** as the spectral information shows (Tables 1 and 2), but

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Table 1
^{1}H NMR data for compounds **6** and **7** (500 MHz)

6		7	
Proton	δ H (mult. in Hz)	Proton	δH (mult. in Hz)
2	7.94 (*s*)	2	7.96 (*s*)
3	–	3	–
8	6.45 (*s*)	8	6.45 (*s*)
3′	6.52 (*s*)	3′	6.52 (*s*)
6′	6.75 (*s*)	6′	6.75 (*s*)
4″	6.27 (*d*, 10.1)	4″	6.27 (*d*, 9.6)
5″	5.52 (*d*, 10.1)	5″	5.52 (*d*, 9.6)
4‴a	2.78 (*dd*, 5.4, 16.9)	1‴a	3.04 (*dd*, 7.3, 15.5)
4‴b	2.99 (*dd*, 5.0, 16.9)	1‴b	3.38 (*dd*, 9.6, 15.5)
5‴	3.90 (*bt*, 5.4)	2‴	5.37 (*dd*, 7.3, 9.2)
7″Me	1.43 (*s*)	7‴	1.43 (*s*)
8″Me	1.43 (*s*)	8″	1.43 (*s*)
7‴Me	1.37 (*s*)		–
8‴Me	1.41 (*s*)		–
2′OH	8.31		8.38
5OH	12.61		12.47
7OH	–		11.70
		3‴	–
		4‴	1.77 (*s*)
		5‴a	4.96 (*s*)
		5‴b	5.11 (*s*)
Solvent	$CDCl_3$		$CDCl_3$

Table 2
^{13} C NMR data for compounds **6** and **7** (125 MHz in $CDCl_3$)

Carbon		**6**	Carbon		**7**
		δ C (mult.)			δ C (mult.)
2		155.3	2		154.7
3		122.9	3		121.6
4		182.2	4		181.9
5		160.1	5		156.6
6		104.2	6		112.0
7		160.3	7		167.0
8		95.2	8		89.1
9		156.1	9		155.6
10		104.9	10		106.0
1′		112.4	1′		109.6
2′		157.2	2′		158.3
3′		107.5	3′		107.3
4′		155.8	4′		157.1
5′		115.5	5′		115.5
6′		127.4	6′		127.1
4″		121.5	4″		121.3
5″		129.0	5″		128.7
6″		77.5	6″		76.6
7″		28.4	7″		28.1
8″		28.4	8″		28.1
4‴ sp^3	Ring E	25.7	1‴ sp^3	Side chain on ring A	30.5
5‴ sp^3		68.8	2‴ sp^3		88.5
6‴ sp^3		79.1	3‴ sp^2		142.8
7‴ sp^3		25.1	4‴ sp^3		16.9
8‴ sp^3		22.2	5‴ sp^2		113.0

there are differences in the side chain attached to ring A: as in **6**, there is a benzylic CH_2 group (C-1‴) giving rise to a double doublet of doublets (centred at δ3.04 and δ3.39). This splitting pattern is consistent with a CH–OH group on the adjacent C-2‴ carbon. From the C-2‴ carbon the chain continues to a C(Me)=CH_2 moiety. The geminal vinyl protons 5‴a and 5‴b at δ4.96 and δ5.11, respectively) take the place of the fourth methyl group present in all the other compounds of the series (Tables 1 and 2). There is no doubt that in **7** there is only

one vinylic methyl group (δ1.77) and it is attached to C-3‴. The chemical relationships of the protons in the side chain are confirmed by clear cut HMQC correlations.

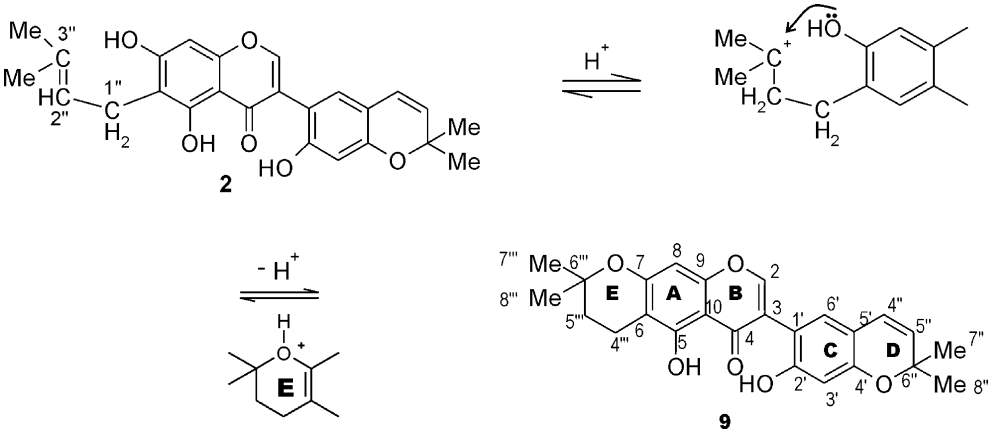
The mass spectrum of **7** fails to show the anticipated molecular ion peak at m/z 436. However, the base peak at m/z 219 (accurate mass $C_{12}H_{11}O_4$) supports the proposed structure. This fragment peak could arise from the ion shown in **8** (Scheme 2).

The nature of the side chain in **7** is somewhat reminiscent of the side chain present in griffonianone D, a metabolite from the root bark of *Millettia griffoniana* (Yankep et al., 2003). In this instance the chain on ring A is $-OCH_2 \cdot CH{=}C(Me)CH_2CH_2CH(OH)C(OH)Me_2$. The 2-hydroxy-3-methyl-3-butenyl substituent is also present in some other prenylated flavonoids (Stevens et al., 2000).

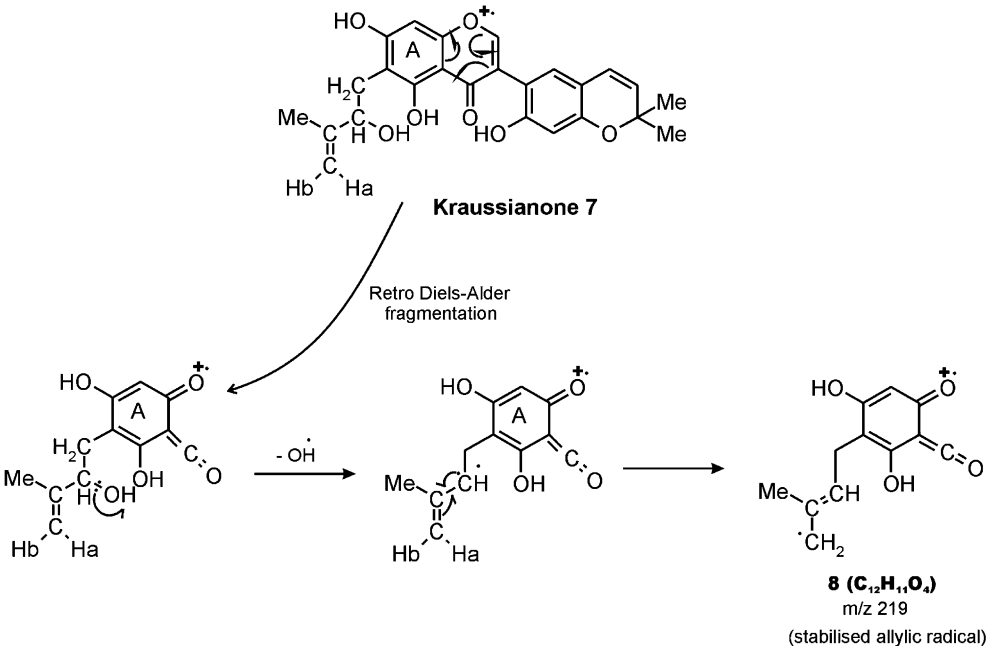
2.1. Chemical reactions

Kraussianone **2** was the major component found in *E. kraussianum* (Drewes et al., 2002) thus affording the opportunity for some chemical studies on it. Could one, for example, induce ring closure? If this produced compound **1**, it would provide a ready route to the compound with the highest E-D activity. Our attempted conversion, using acid catalysis in boiling toluene, afforded not **1**, but **9**. Formation of **9** is in fact the logical end result as the presence of the double bond (C-2″–C-3″) in **2**, facilitates formation of the stable carbocation for attack by the phenolic OH group on C-7 (Scheme 1).

The spectral data for the atoms comprising ring A–D for **9** differ very little for those found in **6** and **7** (see Section 3) as can be anticipated. The adjacent methylenes



Scheme 1. Conversion of **2**–**9**.



Scheme 2. Possible fragmentation of **7**–**8**.

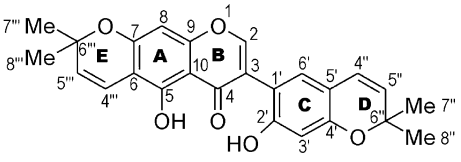
at C-4‴ and C-5‴ resonate as simple triplets and this is not unexpected since **9** is produced by a procedure which renders formation of diastereotopic protons impossible.

2.2. Biological screening

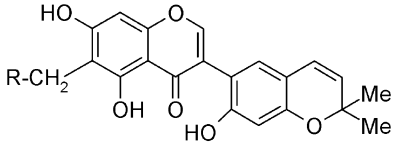
The group of plants belonging to the genus *Eriosema* (traditionally called 'uBangalala' in isiZulu) are used for treating impotence and urinary complaints in males. In practice hot milk infusions of the roots are taken in small doses at night and in the morning.

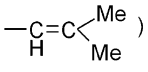
The typical test for a compound which alleviates erectile-dysfunction (E-D) is that done on rabbit *Corpus cavernosum*. If the test compound induces relaxation of the muscle, this is an indication of E-D activity.

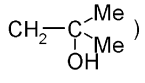
In our first communications on pyrano-isoflavones (Drewes et al., 2002, 2003), we showed that kraussianones **1** and **2** caused *relaxation* of rabbit *Corpus cavernosum*. Relaxation is indicative of E-D activity. Similar tests on kraussianones **3** and **5** were incomplete at the time of publication but preliminary indications were that no relaxation of smooth muscle took place. The full test results for compounds **3** and **5** are now shown in Table 3. Test conditions were identical to those described for compounds **1** and **2** and in this instance Viagra was again used as a control.



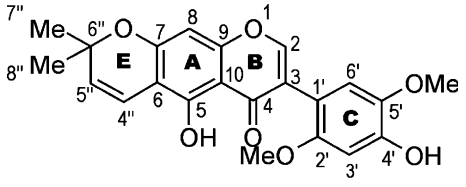
Kraussianone **1**



Kraussianone **2** (R= )

Kraussianone **3** (R = )

To our surprise **3** and **5** caused significant *contraction* of rabbit *C. cavernosum* tissue (Table 3). Tests were carried out at two concentrations. At 138 ng/ml kraussianone **3** produced a contraction of 50% compared with Viagra which showed 100% relaxation. At 78 ng/ml **3** attained a 30% contraction and **5** was less effective at both concentrations. Two interesting observations flow from these results: (i) The whole extract of *E. kraussianum* contains two pyrano-isoflavones (**1** and **2**) capable of causing relaxation of smooth muscle, and two (**3** and **5**) which bring about contraction. Presumably then, the overall effect of the extract, as used in traditional medicine is mainly determined by the properties of **1** and **2** which are present as the major constituents. (ii) We have discovered that Smalberger et al. (1975), working on the root constituents of the indigenous medicinal plant *Tephrosia elongata* (also belonging to the family Papilonaceae) isolated the isoflavone elongatin **10**. In an earlier paper Smalberger et al. (1974) quote from older literature (Watt and Breyer-Brandwijk, 1962) that "*Tephrosia lupinifolia* is used in Barotseland for initiating abortion. A concoction is drunk and is said to kill the foetus. Uterine pains are said to come on in about 10 h". In modern parlance, the concoction is an abortifacient and causes severe contraction of the uterus.



10

What is interesting is that the structure of elongatin **10**, is identical to our compound **1** in terms of rings A, B and E. The dimethylpyran ring D is, however, absent. It is tempting to conclude from the above that the use of isoflavones in traditional reproductive practices now finds support based on current scientific findings.

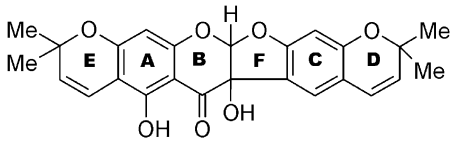
2.3. X-ray crystallography of compound 5

Kraussianones **4** and **5** differed from the other pyrano-isoflavones described here in having an extra ring (F) fused to ring B. Although the spectral evidence fully supported our proposed structure, some doubt remained for 2 reasons:

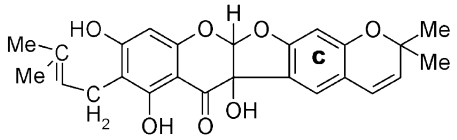
Table 3
Percentage contraction of cavernosal smooth muscle by kraussianones **3** and **5**, Viagra[a] (relaxation)

Kraussianone **3**		Kraussianone **5**		Viagra
78 ng/ml	138 ng/ml	78 ng/ml	138 ng/ml	78 and 138 ng/ml
30.4	50	17.4	23.3	100

[a] The responses by Viagra are treated as 100%.

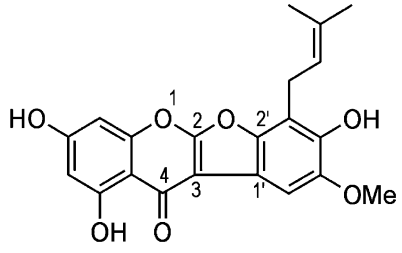


Kraussianone **4**

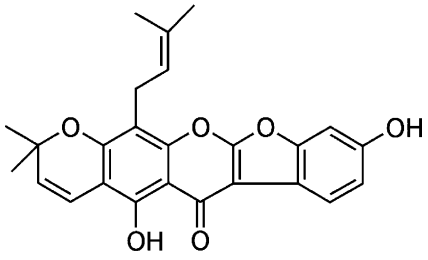


Kraussianone **5**

(1) Compounds **4** and **5**, incorporating a furan ring in the isoflavone system, have not been found previously in Nature whereas the closely related coumarano (2′,3′:2,3) chromone skeleton exists, as for example in lisetin **11** (Falshaw et al., 1966) and milletin **12** (Subba-Raju et al., 1981). In order to distinguish rapidly between a typical isoflavone, coumarano-chromones (as in **11** and **12**) and dihydrochromeno-chromenones (as in **4** and **5**) the chemical shift of the proton on C-2 plays a decisive role. In isoflavones it resonates far downfield (typically at ca. $\delta 8$) it is absent in coumarano-chromones, and in **4** and **5** it appears at about $\delta 6$. In the absence of X-ray data we speculated (Drewes et al., 2002) that this relatively far downfield position of the peak was due to the presence of two geminal ether oxygens. Spectral data supported this but we had no chemical proof.



lisetin **11**



Milletin **12**

(2) Considering the nature of the substituents on C-2 and C-3 in our proposed structures for **4** and **5**, we expected rapid dehydration would occur under the appropriate conditions. We were, however, not able to effect loss of water (heat, acid). In these circumstances we were happy to observe that the X-ray information, when it became available, confirmed the proposed structures despite the lack of chemical proof. With the hindsight of now knowing the relative configuration of the proton on H-2 and the OH on C-3 from the X-ray data, it is clear that the observed *syn* arrangement of these substituents does not favour the elimination of water. It is well known that loss of water is favoured mechanistically if the two substituents are *trans* to one another.

Further study of the solid-state conformation of **5** (Fig. 1) reveals that it has the overall shape of the letter "C" whereas kraussianone **1** in the solid state (Drewes et al., 2002) has the five rings roughly in a straight line but with the possibility of some rotation along the C-3/C-1′ bond. Overall shape (in the preferred conformation), will, of course, influence interaction with a receptor.

The compound **5**, used for X-ray studies, crystallized slowly from hexane–ether and had m.p. 172 °C.

3. Experimental

3.1. General

H, ^{13}C, ^{1}H–^{1}H COSY, DEPT, HSQC and HMQC spectra were run on a Varian 500 MHz spectrometer. High resolution mass spectra came from a Kratos MS 80 RF double focusing magnetic instrument at 70 eV. X-ray studies were carried out on an Oxford Xcalibur 2 CCD diffractometer, (Mo Kα) radiation.

3.2. Plant material

E. kraussianum (rootstock) was collected in October 2002 from the same grassland used previously (Drewes et al., 2003). At this time of the year the plant is flowering and its identity was confirmed by Dr. T. Edwards, curator of the Bews Herbarium at the University of Natal, Pietermaritzburg. The voucher specimen (S.E.D. No. 7) was deposited in the herbarium.

3.3. Extraction and isolation

Dried, milled roots (800 g) were extracted with CH_2Cl_2 at room temperature for 2 days to afford a brown powder (7 g). Some of this crude extract (3.1 g) was separated on a Silica gel 60 column (70–230 mesh ASTM) (4 cm × 1.5 cm) and eluted successively with $CHCl_3$, CH_2Cl_2 and finally ether. The isoflavones were concentrated mainly in the CH_2Cl_2 fraction (510 mg). Subsequent separation of this fraction on a smaller silica gel column (CH_2Cl_2/MeOH, 96:4) followed by purification of the fraction richest in isoflavones (tlc) by the

chromatotron (CH_2Cl_2–MeOH, 96:4) afforded the following compounds in various degrees of purity:

(i) 27 mg of **1** (some contamination with **4** and **7**);
(ii) 190 mg of **2**;
(iii) 47 mg of **3**;
(iv) 23 mg of **5** mixed with traces of **6**.

Careful fractionation of mixed fractions (i) and (iv) finally resulted in the isolation of pure **6** (3.1 mg) and **7** (4.5 mg).

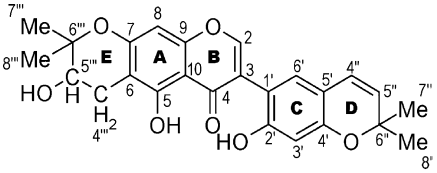
The solvent system above effects a satisfactory separation of the seven components, and the R_f values (below) indicate that **1**, **4** and **7** are the least polar compounds, **2**, **5** and **6** are of medium polarity, while **3** (tert OH in side chain) is the most polar. The R_f values are: 0.77 (**1**, **7**); 0.72 (**4**); 0.62 (**6**); 0.58 (**2**); 0.44 (**5**); 0.14 (**3**).

Isolation and characterization of kraussianones **1**–**5** has been described previously (Drewes et al., 2002).

3.4. Kraussianone (6). 3,5-Dihydroxy-7-(7-hydroxy-2, 2-dimethyl-2H-chromene-6-yl)-2,2-dimethyl-3,4-dihydro-2H,6H-pyrano[3,2 g]chromen-6-one

(The above is the systematic name, but in order to facilitate cross-referencing to the pyrano-isoflavone previously described, the ''older'' numbering has been retained in the text).

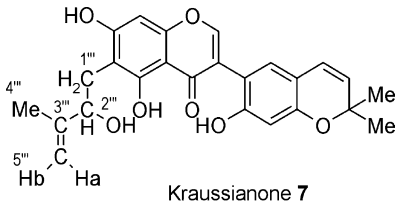
Compound **6** is a light brown solid. The ^1H and ^{13}C spectral data (500 and 125 MHz, respectively, $CDCl_3$) are in Tables 1 and 2, respectively; H-R EI-MS m/z 436.15346 M^+, calcd. for $C_{25}H_{24}O_7$ 436.15220, EI-MS *m/z* (rel. int.): 436 [M^+] (28), 421[M^+-15] (100), 361 (8), 237 (15), 201 (13), 185 (11).



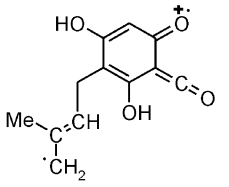
Kraussianone **6**

3.5. Kraussianone (7). 5,7-Dihydroxy-3-(7-hydroxy-2, 2-dimethyl-2H-chromene-6-yl)-6-(2-hydroxy-3-methyl-3-butenyl)-4H-chromen-4-one

Compound **7** is a reddish brown solid. The ^1H and ^{13}C spectral data (500 and 125 MHz respectively, $CDCl_3$) are in Tables 1 and 2 respectively. No molecular ion peak detected by H-R EI-MS. Fragment peak *m/z* 219.06557, (fragment **8**), calcd. for $C_{12}H_{11}O_4$ 219.06573, EI-MS *m/z* (rel. int): 201 (65), 187 (19), 149 (21), 81 (19).



Kraussianone **7**



8 ($C_{12}H_{11}O_4$)

3.6. Conversion of (2) to (9)

To pure compound **2** (20 mg) in toluene (15 ml) 85% H_3PO_4 (2 drops) was added and the solution refluxed for 4 h. The solvent was removed, the mixture purified by chromatotron using hexane/CH_2Cl_2 (1:2) as eluent, and afforded 5.5 mg of solid material.

^1H NMR ($CDCl_3$, 500 MHz): δ1.38 (6H, *s*, 7‴/8‴ Me), 1.43 (6H, *s*, 7″/8″ Me), 1.86 (2H, *t*, $J = 6.4$ Hz, H-5‴), 2.74 (2H, *t*, $J = 6.4$ Hz H-4‴), 5.50 (1H, *d*, $J = 9.1$ Hz, H-5″), 6.28 (1H, *d*, $J = 9.1$ Hz, H-4″), 6.41 (1H, *s*, H-8), 6.53 (1H, *s*, H-3′), 6.76 (1H, *s*, H-6′), 7.93 (1H, *s*, H-2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ16.1 (C-4‴), 26.7 (C-7‴/8‴), 28.1 (C-7″/8″), 31.6 (C-5‴), 76.7 (C-6‴), 76.8 (C-6″), 94.9 (C-8), 104.3 (C-6), 106.0 (C-10), 107.3 (C-

Table 4
X-ray data for compound **5**

Molecular formula	$C_{25}H_{24}O_7$
Formula weight	436
Temperature (K)	295 (2) K
Wavelength (Å)	0.71013
Crystal system	Triclinic
Space group	*P*
Unit cell dimensions	
a (Å)	10.8702 (15)
b (Å)	11.049 (2)
c (Å)	11.582 (2)
Volume (Å)	1316.9 (4)
Z	2
F(100)	544
Crystal size (mm^3)	0.65×0.55×0.45
Reflections collected	5423
Independent reflections	4621 [$R_{(int)} = 0.0080$]
Completeness to $\theta = 24.98°$ (%)	99.7
Maximum and minimum transmission	0.9592 and 0.9418
Goodness-of-fit on F^2	1.025
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0363$, $wR_2 = 0.0962$
R indices (all data)	$R_1 = 0.0477$, $wR_2 = 0.1040$

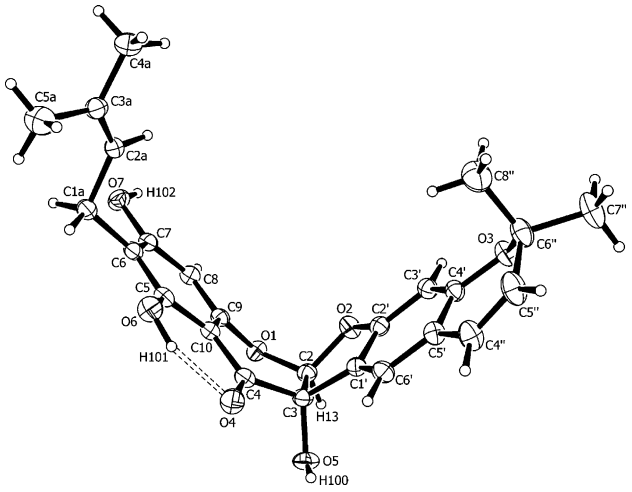


Fig. 1. ORTEP diagram (20% ellipsoids) of compound **5**. The solvent molecule (diethyl ether) has been omitted for clarity.

3′), 112.4 (C-1′), 115.2 (C-5′), 121.3 (C-4″), 122.6 (C-3), 127.1 (C-6′), 128.7 (C-5″), 154.8 (C-2), 155.7 (C-4′/9), 157.1 (C-2′), 159.2 (C-5), 161.3 (C-7), 182.0 (C-4).

3.7. Biological test results on **3** and **5**

The bioassay procedure has been described in our earlier communication (Drewes et al., 2002). Test results (contraction of smooth muscle) are shown in Table 3.

3.8. X-ray data for **5**

Crystal structure data are collected in Table 4 and the solid state structure is in Fig. 1.

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