containing PtO_2 (40 mg) as catalyst was hydrogenated at atm. pres. and room temp. overnight. Usual work-up of the reaction product afforded **1**-acetate (10 mg, mp 78–80°). All the chromatographic and spectral data of the synthetic **1**-acetate were essentially identical with those of naturally occurring **1**-acetate.

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OCCURRENCE OF 24-ETHYL-Δ^5- AND 24-ETHYL-Δ^7-STEROLS AS C-24 EPIMERIC MIXTURES IN SEEDS OF *CUCUMIS SATIVUS*

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Key Word Index—*Cucumis sativus*; Cucurbitaceae; seeds; 24-ethylsterols; spinasterol; chondrillasterol; sitosterol; clionasterol; 22-dihydrospinasterol; 22-dihydrochondrillasterol.

Abstract—^1H NMR and ^{13}C NMR spectroscopy have demonstrated that the 24-ethyl-5α-cholesta-7, *trans*-22-dien-3β-ol, 24-ethylcholest-5-en-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol isolated from the seeds of *Cucumis sativus* are mixtures of both 24α- and 24β-epimers. This seems to be the first instance of the detection of 24β-ethylcholest-5-en-3β-ol in a higher plant.

24β-Ethyl-5α-cholesta-7,22,25(27)-trien-3β-ol (**1**) (all the sterols possessing Δ^{22}-double bond described here have a *trans*-configuration at C-22) and 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol (**2**) together with 24α-ethyl-5α-cholesta-7,22-dien-3β-ol (spinasterol, **3**) and/or its 24β-epimer (chondrillasterol, **4**) are the major sterols in the seeds of some Cucurbitaceae [1]. We have shown recently that a minor 24-methylsterol isolated from the seeds of *Cucumis sativus* is 24α-methyl-5α-cholesta-7,22-dien-3β-ol (stellasterol, **5**) [2]. Our continuing study of the sterols of *C. sativus* seeds has now led to the isolation of three 24-ethylsterols, 24-ethyl-5α-cholesta-7,22-dien-3β-ol, 24-ethylcholest-5-en-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol, and the demonstration that these sterols are mixtures of the epimers at C-24, i.e. **3** and **4**, 24α-ethylcholest-5-en-3β-ol (sitosterol, **6**) and its 24β-epimer (clionasterol, **7**), and 24α-ethyl-5α-cholest-7-en-3β-ol (22-dihydrospinasterol, **8**) and its 24β-epimer (22-dihydrochondrillasterol, **9**), respectively. The other sterols identified were **1** and **2** in addition to **5**.

The sterol fraction that was separated from the unsaponifiable lipid of *C. sativus* seed oil was acetylated, and the resulting acetate fraction was separated into four bands (refered to as bands 1–4 in order of polarity, beginning with the least polar) by silver nitrate-silica gel TLC [2]. The fraction recovered from band 1 (R_f 0.66) was hydrolysed and the resulting free sterol fraction was subjected to reversed-phase HPLC (ODS-2 column), which yielded the mixture (RR_t 1.18 in HPLC) of 24-ethylcholest-5-en-3β-ol (sterol A, RR_t 1.61 in GC, **6**

Table 1. Methyl group ^{1}H NMR chemical shifts (400 MHz, $CDCl_3$) of some 24-ethyl-Δ^5- and 24-ethyl-Δ^7-sterols*

	C-18	C-19	C-21	C-26	C-27	C-29
:rol (24α, **6**)	0.680 (*s*)	1.009 (*s*)	0.921 (*d*, *J* = 6.6)	0.835 (*d*, *J* = 7.3)	0.813 (*d*, *J* = 6.8)	0.844 (*t*, *J* = 7.6)
isterol (24β, **7**)	0.680 (*s*)	1.009 (*s*)	0.925 (*d*, *J* = 6.4)	0.833 (*d*, *J* = 6.9)	0.813 (*d*, *J* = 6.8)	0.854 (*t*, *J* = 7.4)
A	0.680 (*s*)	1.009 (*s*)	0.921 (*d*, *J* = 6.4) 0.925 (*d*, *J* = 6.4)	0.833 (*d*, *J* = 7.3)	0.815 (*d*, *J* = 6.8)	0.844† (*t*, *J* = 7.8) 0.854† (*t*, *J* = 7.6)
ıydrospinasterol (24α, **8**)	0.536 (*s*)	0.796 (*s*)	0.926 (*d*, *J* = 6.3)	0.837 (*d*, *J* = 7.1)	0.815 (*d*, *J* = 6.8)	0.846 (*t*, *J* = 7.8)
ıydrochondrillasterol (24β, **9**)	0.535 (*s*)	0.796 (*s*)	0.931 (*d*, *J* = 6.6)	0.832 (*d*, *J* = 6.8)	0.811 (*d*, *J* = 6.8)	0.855 (*t*, *J* = 7.3)
B	0.535 (*s*)	0.796 (*s*)	0.925 (*d*, *J* = 6.4) 0.930 (*d*, *J* = 6.4)	0.833 (*d*, *J* = 6.8)	0.813 (*d*, *J* = 7.3)	0.844‡ (*t*, *J* = 7.8) 0.854‡ (*t*, *J* = 7.3)

l chemical shifts given in δ values from TMS; coupling constants in Hz.
lative height of the lower-field resonance peak of the triplet at δ0.844 and 0.854 was 8:2.
lative height of the lower-field resonance peak of the triplet at δ0.844 and 0.854 was 7:3.

ır **7**) and 24-ethyl-5α-cholest-7-en-3β-ol (sterol B, .88 in GC, **8** and/or **9**). Further separation of the ıre was performed by HPLC, affording A (mp 44°) and B (mp 135–138°). The separation factor of ^{5}/Δ^7-24-ethylsterols (A/B) on the HPLC reversed column was 1.03. The 400 MHz ^{1}H NMR spectra recorded for these sterols and two pairs of authentic ıylsterols with different stereochemistry at C-24, i.e. ıyl-Δ^5-sterols, **6** (24α) and **7** (24β), and 24-ethyl-Δ^7-s, **8** (24α) and **9** (24β), in order to determine the guration at C-24 of sterols A and B. Table 1 shows ıemical shifts of the methyl group signals for which ıments were made by comparison with literature [3–5]. As can be seen from Table 1, the most inent difference in the chemical shift was observed ıe C-29 methyl triplet [$\Delta\delta$(24β-24α) = 0.009–0.010] ıen each pair of diastereoisomeric 24-ethylsterols, i.e. **7**, and **8** and **9**, respectively, as has been recognized ıusly [4, 5]. Thus, an inspection of the spectra of s A and B revealed that both sterols were mixtures of -24 epimers. Based on the relative heights of the -field resonance peak present in the C-29 proton t [5] arising from each of the two diastereoisomers in ixture, the relative ratio was estimated as **6** (24α): **7** = 8:2 for sterol A, whereas **8** (24α):**9** (24β) = 7:3 for B.

e fraction from band 2 (R_f 0.56) of the silver nitrate-gel TLC contained the acetates of **5** [2] and 24-ethyl-olesta-7,22-dien-3β-ol (**3** and/or **4**). The latter (mp .81°) was shown by ^{13}C NMR spectroscopy [1], isolation by further TLC on silver nitrate–silica gel, ıixture of the sterols epimeric at C-24 with a relative of **3**:**4** = 4:6. The fraction from band 3 (R_f 0.49) and rom band 4 (R_f 0.11) afforded the acetates of **2** (mp .63°) and **1** (mp 175–177°), respectively. The 24β-guration of **1** and **2** was verified by direct comparison eir ^{13}C NMR spectra with those of authentic com-ds. The quantitative composition of the total sterol on of *C. sativus* seeds was determined from the GC, ^{1}H NMR and ^{13}C NMR data as 65.8% (**1**), 16.4% (**2**), 1.6% (**3**), 2.4% (**4**), 2.2% (**5**), 1.2% (**6**), 0.3% (**7**), 2.3% (**8**), 1.0% (**9**) and 6.8% of several unidentified minor sterols. GC and mass spectral data of the identified sterols were consistent with those of the authentic compounds.

Thus, the co-occurrence of both C-24 epimers of 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**3** and **4**), 24-ethyl-cholest-5-en-3β-ol (**6** and **7**) and 24-ethyl-5α-cholest-7-en-3β-ol (**8** and **9**) was demonstrated in the seeds of *C. sativus*. The co-existence of **3** and **4** in the seeds of *Lagenaria leucantha* var. *gourda* and *Citrullus battich* [1], and **8** and **9** in the roots of *Trichosanthes japonica* [6] has recently been shown. Although clionasterol (**7**) is known as a component sterol in green algae [7], this seems to be the first instance of the detection of **7** in a higher plant.

EXPERIMENTAL

Mps are uncorr. HPLC was carried out on a Partisil 5 ODS-2 column (Whatman, 20 cm × 6 mm i.d.; packed by Erma Optical Works, Tokyo) and an Altex Ultrasphere ODS 5 μm (25 cm × 10 mm i.d.) using a UV detector monitoring at 214 nm (mobile phase, MeOH–H_2O, 49:1). GC equipped with a hydrogen-flame ionization detector was performed on a glass column (2 m × 3 mm i.d.) containing 3% OV-17/Gas Chrom-Q (carrier gas N_2, temp. 260°). RR_t in HPLC and GC were expressed relative to cholesterol. $AgNO_3$-silica gel (1:4) TLC (0.5 mm) was developed 4 × with CH_2Cl_2–CCl_4 (1:5). MS (70 eV) were taken with a direct inlet system. ^{1}H NMR (400 MHz) and ^{13}C NMR (25.05 MHz) spectra were determined in $CDCl_3$ with TMS as int. standard. Origin of the seed material [8], isolation of the sterol fraction from the seeds [2], and our general techniques [1] have been described previously. Sterols **1**–**4**, **8** and **9** [1], **5** [2], and **6** and **7** [9] were used as the authentic samples. The ^{13}C NMR spectra of the acetates of **1**–**4** were shown in the previous article [1].

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WAMPETIN, A FUROCOUMARIN FROM *CLAUSENA WAMPI*

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Key Word Index—*Clausena wampi* (Syn. *Clausena lansium*); Rutaceae; wampetin; furocoumarin; ^{13}C NMR data.

Abstract—A new furocoumarin wampetin has been isolated from *Clausena wampi* (syn. *Clausena lansium*). The structure was established from ^1H NMR, ^{13}C NMR, MS and chemical data.

The aerial and underground parts of *Clausena* species have been studied extensively for the presence of coumarins [1, 2] and carbazoles [3]. Here we report the isolation and characterization of a new furocoumarin (**1**), wampetin, from the root bark of *Clausena wampi* Blanco (Syn. *C. lansium*)†.

Wampetin (**1**), mp 78°, analysed for $C_{21}H_{18}O_6$ ($[M]^+$ 366). It showed IR bands at 1755 cm^{-1} and 1710 cm^{-1} indicative of the presence of an α,β-unsaturated-γ-lactone and α,β-unsaturated-δ-lactone groups. Its cleavage with concentrated sulphuric acid afforded Xanthotoxol [4] indicating that wampetin is a C-8 ether of xanthotoxol. The 200 MHz ^1H NMR spectrum of **1** integrated for 18 protons and the assignments of their chemical shift values are given in Table 1. The proposed structure was supported by decoupling experiments (NMDR technique) and ^{13}C NMR data.

EXPERIMENTAL

The EtOAc extract of powdered and dried root bark of *C. wampi* on CC followed by prep. TLC afforded wampetin (**1**) from EtOAc–Et_2O, mp 78° (uncorr.). MS *m/z* (rel. int.): 366 (3.2), 202

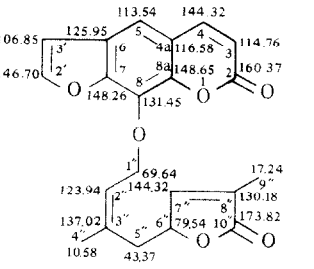

Table 1. ^1H NMR spectral data of compound **1** (200 MHz, $CDCl_3$, TMS as int. stand.)

H	δ	H	δ
2′	7.71 (1H, *d*)	2″	5.72 (1H, *tm*)
3′	6.83 (1H, *d*)	4″	1.79 (3H, *s br*)
3	6.35 (1H, *d*)	5″	2.36 (2H, *m*)
4	7.79 (1H, *d*)	6″	4.92 (1H, *tm*)
5	7.39 (1H, *s*)	7″	6.93 (1H, *dq*)
1″	5.09 (2H, *d*)	9″	1.88 (3H, *t*)

J-values in Hz: $J_{H\text{-}2',H\text{-}3'} = 2.30$; $J_{H\text{-}3,H\text{-}4} = 9.52$; $J_{H\text{-}1'',H\text{-}2''} = 6.6$; $J_{H\text{-}2'',H\text{-}4''} = 0.98$; $J_{H\text{-}5'',H\text{-}6''} = 6.5$; $J_{H\text{-}6'',H\text{-}7''} = 1.6$; $J_{H\text{-}7'',H\text{-}9''} = 1.71$; $J_{H\text{-}6'',H\text{-}9''} = 1.95$.

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†Collected from Forest Research Institute, Dehradun and identified by Mr. Kunwar Naresh Bahadur, Botany Division.