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TEPANONE, A RETROCHALCONE FROM *ELLIPEIA CUNEIFOLIA*

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Key Word Index—*Ellipeia cuneifolia*; Annonaceae; roots; stembark; chalcone; phenylpropenoid; retrochalcone; tepanone; methyltepanone; *trans–cis* isomerisation.

Abstract—Tepanone, (2*E*)-1-phenyl-3-(2-hydroxy-3,4,6-trimethoxyphenyl)prop-2-enone was isolated from roots and stembark of *Ellipeia cuneifolia*. The structure was deduced from spectroscopic data and confirmed by synthesis of the methylated derivative, methyltepanone. Methyltepanone exists as the two readily interconvertible *trans* and *cis* isomers.

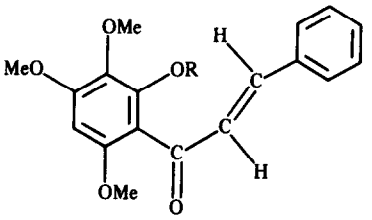
INTRODUCTION

Ellipeia cuneifolia Hook, F. & Thoms. is a small shrub 1–2 m in height and is the only species of this genus that is found in the northern region of Peninsular Malaysia [1]. The plant is known locally as 'Tepan' and, according to local herbalists, a decoction of the roots has been used at post-parturition.

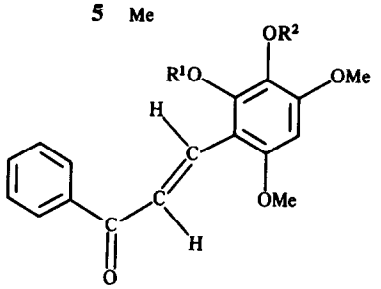
RESULTS AND DISCUSSION

Chromatographic purification of the chloroform extracts of the roots or stembark of *E. cuneifolia* yielded tepanone as yellow needles. A chloroform solution of tepanone rapidly turns dark red in colour, with concomitant formation of by-products, if traces of acid are not removed. The high resolution mass spectral data indicated a molecular formula $C_{18}H_{18}O_5$. A carbonyl entity was readily observed in the ^{13}C NMR spectrum whilst a mono-substituted phenyl ring, a *trans*-substituted olefinic bond and a penta-substituted phenyl ring were each evident from the ^1H NMR spectrum. The penta-substitution included three methoxyl groups and a non-hydrogen bonded hydroxyl. The deshielding of one of the methoxyl carbon resonances (δ61.29) implied that this methoxyl group is flanked by two other bulky substituents, whereas the chemical shifts of the other two methoxyl substituents (δ55.98 and 55.90) indicated that these are *ortho* to the aromatic proton. Furthermore, the evident shielding of the ring carbons which are bonded to the aromatic proton, to one of the methoxyl substituents and to the propenone side chain indicated that these carbons are each flanked by oxygen-bearing carbons [2]. Observation of the base peak at *m/z* 105, in the mass spectrum, indicated a benzonium ion. This seemed to eliminate structures with A ring oxygenation such as **1** which, coincidentally, possesses the same melting point as the isolate described in this report but displays quite different NMR spectral data [3]. Thus, the mass and NMR spectral data suggested structures **2** or **3** for tepanone. The mass spectral fragmentation pattern supported this suggestion by displaying ions indicative of either half of the molecule. The prominent fragment ion corresponding to $[M-31]^+$ has been shown to indicate an *ortho* relationship of a methoxyl substituent to the propenoid side chain [4].

2D C–H HETCOR (correlations through one bond) and COLOC (long range correlations) [5] NMR experiments were used to establish the substitution pattern of the oxygenated ring and to demonstrate the bonding of this ring to the β carbon of the prop-2-enone side chain (Table 1). Note, in particular, how the correlations of the

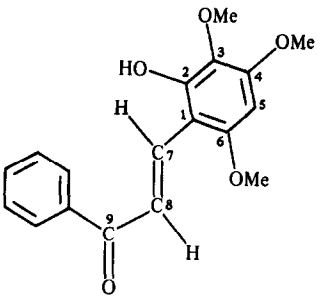


	R
1	H
5	Me



	R^1	R^2
2	Me	H
3	H	Me
4	Me	Me

Table 1. HETCOR and COLOC H–C correlations



Protons (ppm)	Correlated carbons
H-2′, H-6′ (8.23–8.26)*	C-2′, C-6′, C-9
H-7 (8.23)	C-7, C-8, C-9, C-2, C-6
H-8 (7.98)	C-8, C-9, C-7, C-1
OH (6.62)	C-2, C-1, C-3 (weak)
H-5 (6.08)	C-5, C-6, C-4, C-3, C-1
OMe (3.92)	OMe (55.90), C-4[a]
OMe (3.89)	OMe (55.98), C-6[a]
OMe (3.86)	OMe (61.29), C-3

[a]Assignments may be reversed.
*Using benzene-d_6 as solvent.

aromatic proton in the substituted ring establish its relationship with the methoxyl-bearing carbons and how the correlations of the hydroxyl proton and the β olefinic proton effectively lock-in the hydroxyl substituent to the 2 position. Because of the overlap of NMR signals when using chloroform-*d* as a solvent, correlation between the *ortho* protons (2′ and 6′) on the unsubstituted A ring and the carbonyl carbon was confirmed using benzene-d_6 as the solvent which resulted in marked separation of the olefinic and aromatic proton signals. Thus tepanone is (2*E*)-1-phenyl-3-(2-hydroxy-3,4,6-trimethoxyphenyl)-prop-2-enone (**3**).

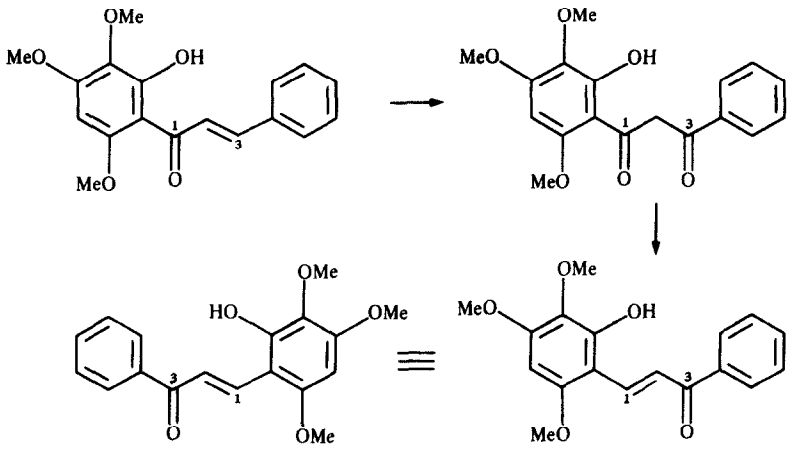
Biosynthetic considerations

With the non-oxygenated A-ring and a phloroglucinol oxygenation pattern on the B-ring, tepanone clearly belongs to the retrochalcone class of compounds [4, 6]. The first example of this class was echinatin (**6**), a constituent of the tissue culture of *Glycyrrhiza echinata* [7, 8]. Radiotracer experiments provided evidence that ring A of echinatin originates from cinnamoyl CoA, whereas ring B is formed from the acetate-malonate pathway [9, 10]. It has been suggested that the retrochalcones derive from the normal chalcones via the intermediate 1,3-dicarbonyl compound which could then undergo reduction of the carbonyl initially at C-1 and subsequent dehydration [9, 10]. The essential features of this 1,3-carbonyl transposition are illustrated in Scheme 1 for the formation of tepanone.

Methylation and trans–cis *isomerisation*

As further proof of the retrochalcone nature of this compound, the NMR spectroscopic data of methyltepanone (**4**) were quite different to those of the tetramethoxy chalcone (**5**) prepared by Panichpol and Waterman [3]. During purification of the methylation product it was observed, by TLC, that another compound of slightly lower R_f was formed when a solution of methyltepanone was exposed to light. To investigate this transformation, methyltepanone (**4**) was prepared via the Claisen–Schmidt condensation of acetophenone with 2,3,4,6-tetramethoxybenzaldehyde. The synthetic compound was identical in all respects, including the observed transformation, to the methylated natural product.

A solution of the synthetic methyltepanone or the methylated natural product (compound A) in chloroform soon began to form another compound (compound B), evident as a slightly lower spot on TLC. The retention times of these compounds were reversed under the GC-MS conditions, compound B being eluted before compound A. The mass spectra of both compounds were identical and both indicate methyltepanone (**4**). This evident isomerisation was monitored by TLC, GC-MS and by ^{1}H NMR spectroscopy. The NMR spectrum, of a solution of A allowed to equilibrate over 72 hr, showed signals for both isomers with isomer B predominating. When a minute drop of concentrated HCl was then added to this solution, the colour immediately darkened from yellow to orange. The NMR spectrum of this acidified solution, dried by passing the solution through sodium



Scheme 1.

sulphate, showed one set of signals and the GC-MS showed only one peak corresponding to isomer A. Isomer A does not change to isomer B if kept in the dark.

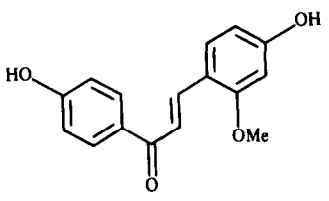
The most significant difference between the ^{1}H NMR spectra of the isomers was in the chemical shifts and the coupling constants of the α and β protons of the propenone entity. In isomer A, these protons are deshielded, as expected in proximity to the benzoyl group, with a coupling constant of 15.9 Hz. However, in isomer B these protons shifted considerably upfield and the coupling constant decreased to 12.1 Hz, indicating a *trans* to *cis* isomerisation. This indication was confirmed by recording the NOE difference NMR spectra [11] of both isomers in benzene-d_6 solution. As expected, both isomers showed NOE enhancement of the signal for the aromatic proton on ring B when the methoxyl groups at C-4 and C-6 were irradiated. More importantly, however, isomer B showed a NOE between the α and β protons of the propenone system, thereby establishing it as the *cis* isomer. Isomer A (the *trans*-isomer) showed no such NOE between the α and β protons.

The *trans–cis* isomerisation of A to B was also monitored by UV absorption spectroscopy. The absorption peak at 370 nm in the UV spectrum of isomer A moved to shorter wavelength (350 nm) and decreased in intensity for isomer B. This hyperchromic, bathochromic shift is analogous to that observed in the UV spectrum of *trans*-benzalacetophenone as it isomerises to the *cis* isomer under the same conditions as the methyltepanone isomerisation [12].

This relative ease of *trans–cis* isomerisation may explain the observed instability of tepanone. Thus the *cis*-isomer (7) can produce the 2-hydroxy-flav-3-ene (8) which can generate the coloured flavylium salt on contact with traces of acid. It should be noted that some uncertainty in the melting point of aged samples, presumed to be isomerically pure, could be a consequence of the rather facile *trans–cis* isomerisation observed for this type of compound.

EXPERIMENTAL

General experimental details were as reported previously [13]. The plant was collected at Kelantan, Peninsular Malaysia and a voucher specimen is lodged with the Herbarium at Universiti Kebangsaan Malaysia (A. Latiff, ALM 3142).



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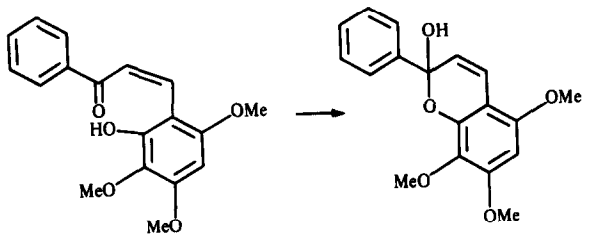

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Isolation of tepanone (3). The dried, powdered roots of *E. cuneifolia* (330 g) were extracted with $CHCl_3$ for 72 hr under Soxhlet conditions. The extract was filtered and the solvent evapd to yield a viscous, brown residue (6.41 g). Adsorption of this onto silica gel (230–400 mesh) and elution with $CHCl_3$ furnished a yellow powder (0.27 g). Recrystallisation from Et_2O yielded tepanone as yellow needles, mp 140–141°. A similar yield of tepanone was also obtained from the dried, powdered stem bark of *E. cuneifolia* (640 g) following extraction with $CHCl_3$ at room temp. for 72 hr. EI-HRMS found *m/z* 314.1155, $C_{18}H_{18}O_5$ expects 314.1154. ^{13}C NMR ($CDCl_3$): 191.9 (carbonyl), 156.7 (C-6 OMe), 154.1 (C-4 OMe), 150.6 (C-2), 139.1 (C-1'), 135.8 (Cβ), 132.1 (C-4'), 129.7 (C-3 OMe), 128.5 (C-2' and C-6'), 128.4 (C-3' and C-5'), 122.5 (Cα), 105.3 (C-1), 88.2 (C-5), 61.3 (C-3 OMe), 56.0 (C-6 or C-4 OMe), 55.9 (C-4 or C-6 OMe); ^{1}H NMR ($CDCl_3$): 8.23 (1H, *d*, J = 15.9 Hz, Hβ), 8.03 (2H, *m*, H-2' and H-6'), 7.98 (1H, *d*, J = 15.9 Hz, Hα), 7.50 (3H, *m*, H-3', H-4' and H-5'), 6.62 (1H, *s*, OH), 6.08 (1H, *s*, H-5), 3.92 (3H, *s*, C-5 or C-6 OMe), 3.89 (3H, *s*, C-6 or C-5 OMe), 3.86 (3H, *s*, C-3 OMe); EIMS *m/z* 314 (13%, $[M]^+$), 297 (5, $[M-OH]^+$), 283 (46, $[M-OMe]^+$), 237 (1.7, $[M-Ph]^+$), 222 (3, [237 – Me]), 208 (1, $[M-Ph-C{\equiv}O]^+$, H^+), 193 (2, [208 – Me]), 179 (2), 178 (2), 105 (100, $[Ph-C{\equiv}O]^+$), 77 (25, $[Ph]^+$); IR ν_{max}^{KBr} cm^{-1}: 3000 (w), 2940 (w), 2844 (w), 1643 (m), 1610 (m), 1581 (m), 1563 (s), 1511 (m), 1469 (m), 1343 (s), 1238 (m), 1213 (m), 1116 (s), 1048 (m); UV λ_{max}^{EtOH}: 371 nm (log ε 3.93).

Methylation of tepanone. A soln of tepanone (10 mg) in CH_2Cl_2 (5 ml) and DMS (100 μl) was stirred, at room temp. and under N_2, with aq. 0.1 M NaOH (5 ml) in the presence of a phase transfer reagent, tetra-*t*-butylammonium chloride. Once the aq. layer became colourless, indicating complete reaction of the phenolic anion, the reaction mixture was warmed at 50° under vacuum to evaporate the organic solvent and decompose excess DMS. The dried $CHCl_3$ extract, of the resultant aq. suspension, was evapd to yield methyltepanone as a yellow/orange oil (8.5 mg). EI-HRMS found *m/z* 328.1311, $C_{19}H_{20}O_5$ calcd 328.1311. GC-MS 250° isocratic, 25 m 5% diphenyl, 95% dimethylsiloxane capillary column; *trans*-isomer 17 min, *cis*-isomer 8 min. EIMS *m/z*: 328([M], 5%), 297 (100), 267 (16), 253 (5), 293 (5), 105 (25), 77 (20); ^{1}H NMR (C_6D_6): *trans*-isomer, 8.90 (1H, *d*, $J_{\alpha,\beta}$ = 15.9, Hβ), 8.44 (1H, *d*, $J_{\alpha,\beta}$ = 15.9, Hα), 8.29 (2H, *m*, H-2', H-6'), 7.22 (3H, *m*, H-3', H-4', H-5'), 5.95 (1H, *s*, H-5), 3.79 (3H, *s*, OMe), 3.78 (3H, *s*, OMe), 3.36 (3H, *s*, C-6 or C-4 OMe), 3.31 (3H, *s*, C-4 or C-6 OMe). *cis*-Isomer, 8.01 (2H, *m*, H-2', H-6'), 7.03 (4H, *m*, Hβ, H-3', H-4', H-5'), 6.50 (1H, *d*, $J_{\alpha,\beta}$ = 12.6, Hα), 5.76 (1H, *s*, H-5), 3.67 (3H, *s*, OMe), 3.61 (3H, *s*, OMe), 3.21 (3H, *s*, C-4 or C-6 OMe), 3.09 (3H, *s*, C-6 or C-4 OMe); ^{13}C NMR ($CDCl_3$): *trans*-isomer, 191.97 (carbonyl), 156.7 (C-6 OMe), 155.7 (C-4 OMe), 154.7 (C-2 OMe), 139.0 (C-1'), 136.5 (Cβ), 136.0 (C-4'), 132.2 (C-3 OMe), 128.7 (C-2' or C-6'), 128.4 (C-6' or C-2'), 128.4 (C-3' or C-5'), 127.9 (C-5' or C-3'), 123.2 (Cα), 110.9 (C-1), 91.9 (C-5), 61.1 (C-3 OMe), 61.1 (C-1 OMe), 55.9 (C-6 and C-4 OMe). *cis*-Isomer (C_6D_6), 192.0 (carbonyl), 154.66 (C-6 OMe), 153.4 (C-4 OMe), 152.9 (C-2 OMe), 139.5 (C-1'), 137.1 (Cβ), 136.0 (C-4'), 132.0 (C-3 OMe), 123.9 (Cα), 112.9 (C-1), 92.6 (C-5), 60.8 (C-3 OMe), 60.6 (C-1 OMe), 55.5 and 54.7 (C-6 and C-4 OMe); IR ν^{CHCl_3} cm^{-1}: *trans*-isomer, 2960 (m), 2860 (m), 1722 (m), 1663 (m), 1601 (s), 1590 (s), 1575 (s), 1500 (m), 1471 (s), 1410 (m), 1345 (m), 1330 (m), 1120 (s); UV λ_{max}^{EtOH}: *trans*-isomer 257, 371 nm (log ε 4.13, 4.19). *cis*-Isomer 255, 351 nm (log ε 4.16, 3.64).

Synthesis of methyltepanone (4). A 50% aq. soln of KOH (50 μl) was added to a stirred soln of acetophenone (200 mg) and 2,3,4,6-tetramethoxybenzaldehyde (100 mg, prepared by methylation of 2-hydroxy-4,6-dimethoxybenzaldehyde followed by

Baeyer–Villiger oxidation, methylation and Villsmeir–Haack formylation). The reaction was monitored by TLC and was complete within 1 hr. The reaction soln was diluted with water and extracted with $CHCl_3$. The dried organic extract was concd and radially chromatographed to provide the required retrochalcone as a yellow oil (103 mg).

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