Pergamon

0031-9422(95)00969-8

Phytochemistry, Vol. 42, No. 3, pp. 795–798, 1996

0031-9422/96 $15.00 + 0.00

A CYCLOARTANE TRITERPENOID AND ω-PHENYL ALKANOIC AND ALKENOIC ACIDS FROM *TRICHILIA CLAUSSENII*

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(*Received in revised form* 2 *November* 1995)

Key Word Index—*Trichilia claussenii*; Meliaceae; cycloartane triterpenoids; ω-phenyl alkanoic and alkenoic acids.

Abstract—The hexane extract of leaves of *Trichilia claussenii* yielded the new triterpenoid 24-methylene-26-hydroxycycloartan-3-one, 24-methylenecycloartanol fatty acids derivatives, caryophyllene epoxide, a mixture of ω-phenyl alkanoic and alkenoic acids, plastocromenol, α-tocopherol, squalene, and a mixture of sitosterol and stigmasterol as free alcohols and esterified by fatty acids. The methanol extract afforded *N*-methyl-proline, 4-hydroxy-*N*-methylproline, ω-phenyl alkanoic acids and a mixture of β-*O*-D-glucopyranosides of sitosterol and stigmasterol.

INTRODUCTION

Plants of the Meliaceae family have been used to control insect pests. The C-*seco*-limonoids have been shown to have good antifeedant activities. Azadirachtin, belonging to this group, is the most potent antifeedant [1]. These limonoids appear to be restricted to *Melia* and *Azadirachta* species, but heudebolin, a typical member of the group, was found in the bark of *Trichilia heudelotti* [2]. However, it has to be said that there is the possibility that extracts were confused during processing [2]. Clearly, much more detailed phytochemical investigations of *Trichilia* species will be essential. Thus, our interest in antifeedant compounds and particularly our taxonomic interest in the Meliaceae stimulated an investigation of other Brazilian plants of the *Trichilia* genus [3]. In this paper we report a phytochemical study on the leaves of *T. claussenii* DC., which grows in São Paulo State, Brazil.

RESULTS AND DISCUSSION

The leaves of *T. claussenii* were percolated with hexane, methylene chloride and methanol, respectively. The hexane extract after chromatography gave some terpenoids and ω-phenyl alkanoic acids. Compound **1** appeared to be a cycloartane triterpenoid. Its IR spectrum showed absorption due to a ketonic carbonyl (1695 cm^{-1}) and a hydroxyl (3557 cm^{-1}). In the mass spectrum the parent ion observed, *m/z* 424, does not represent the molecular ion which is expected to be *m/z* 454. This means that a McLafferty type rearrangement occurred in the side chain with the loss of a fragment *m/z* 30. Its ^{1}H NMR spectrum showed a shielded AB system (δ 0.55 and 0.76, both *d*, *J* = 4.4 Hz) arising from to two cyclopropane protons; a doublet of doublets of doublets (δ 2.29, *J* = 2.8, 4.4 and 14.0 Hz) for H-2α; a triplet of doublets (δ 2.68, *J* = 6.4, 13.6 and 13.6 Hz) for H-2β; a septet (δ 2.34, *J* = 6.8 Hz) for H-25; two superimposed doublet of doublets (δ 3.49, J = 7.2 and 10.8 Hz; δ 3.51, *J* = 6.4 and 10.6 Hz) for H-26; a broad singlet (δ 4.79) and a broad doublet (δ 4.86), both attributed to H-31 in the side chain. Based on the ^{1}H–^{1}H COSY spectrum it was also possible to assign the doublets (δ 0.88, J = 6.0 Hz) to Me-21 and (δ 1.02, *d*, *J* = 6.8 Hz) to Me-27. This spectrum also showed correlation between H-26 and H-25, H-2β and H-2α besides H-19a with H-19b. Compound **1** was acetylated, yielding the corresponding monoacetate (**1a**) whose ^{1}H–^{1}H COSY spectrum permitted the assignment of all the chemical shifts for the protons in the side chain. The attribution of chemical shifts for the carbons in the side chain of **1a** was achieved by comparison with a model compound from the literature [4]. It seems that this is the first time that compound **1** has been described in the literature.

Compound **2** was also identified as a cycloartane derivative, with a fatty acid esterifying the alcohol at C-3. After hydrolysis of **2** under basic conditions, an alcohol **2a** and a mixture of acids were obtained. The acids, after esterification with diazomethane and GC-mass spectral analysis, were identified as a mixture of methyl esters of fatty acids ranging from C-14 to C-26.

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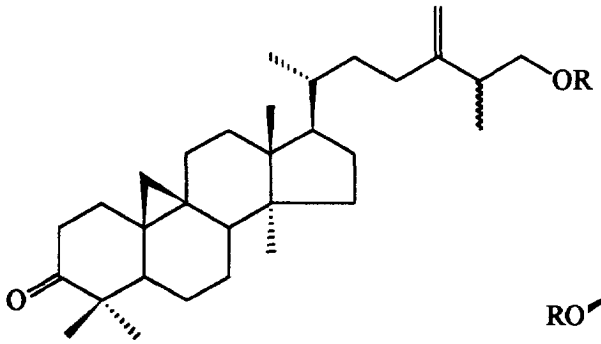
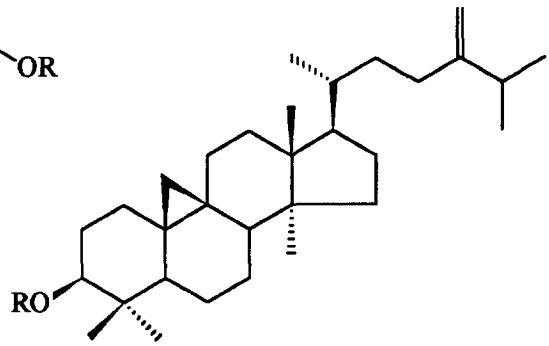
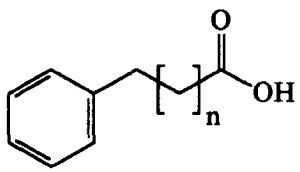


1 R=H
1a R=Ac

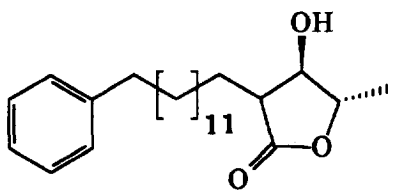


2 R=$CH_3(CH_2)_nCO$
2a R=H



3

3a n=8
3b n=9
3c n=10
3d n=11
3e n=12
3f n=13



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However, palmitic acid was identified as the major compound in this mixture. Compound **2a** was shown to be 24-methylenecycloartan-3β-ol on the basis of its ^{1}H NMR spectrum, which revealed two broad singlets (δ 4.72 and 4.66; 2H-31); a doublet of doublets (δ 3.28, J = 11.0 and 4.0 Hz, H-3); a heptet (δ 2.24; J = 6.8 Hz; H-25); two doublets (δ 0.55 and 0.33, 2H-19). Me-26 and Me-27 appeared as two doublets (δ 1.03 and 1.02, J = 6.8 Hz) and Me-21 as a doublet (δ 0.9, J = 6.0 Hz). Compound **2** had already been described and its spectral data match with those described in the literature [5].

The ω-phenyl alkanoic acids (**3**) were identified in mixture through GC-mass spectral analysis and NMR after esterification with diazomethane. The ^{1}H NMR spectrum showed signals of an aromatic ring (δ 7.14–7.28); a broad triplet (δ 5.34, J = 5.2 Hz), due to olefinic protons of the fatty acids with a double bond in the side chain; two triplets (δ 2.59, J = 7.6 Hz, and δ 2.29, J = 7.6 Hz) attributable to the benzyl and α-carbonyl protons respectively; a singlet (δ 3.65 CO_2Me), and a series of signals (δ 1.0–2.0) for the methylene protons in the side chain. The ^{13}C NMR of **3** confirmed the presence of an ester carbonyl as well as an aromatic ring and a double bond in the side chain. Analysis of the methyl esters of **3** by GC-mass spectrometry allowed us to identify and establish the ratios of each acid in the mixture as: ω-phenyl decanoic (**3a**, 1.12%), ω-phenyl undecanoic (**3b**, 2.45%), ω-phenyl dodecanoic (**3c**, 36.03%), ω-phenyl tridecanoic (**3d**, 39.95%), ω-phenyl tetradecanoic (**3e**, 3.49%) and ω-phenyl pentadecanoic (**3f**, 3.04%). The corresponding alkenoic acids containing 14 (**3g**, 3.30%) and 15 (**3h**, 6.15%) carbon atoms were also identified, though the position of the double bond was not established.

N-Methylproline and 4-hydroxy-*N*-methylproline were identified mainly on the basis of their ^{1}H and ^{13}C NMR spectra through comparison with the literature [6]. Both compounds were methylated with diazomethane to yield the corresponding *N*-dimethyl derivatives. Caryophyllene epoxide was also isolated [7].

The ω-phenyl alkanoic acids can be biosynthesized

through direct condensation of C_6–C_3 units with polyketide chains of different lengths. Grandinolides and other related compounds were known until recently to occur only in the Myristicaceae and Lauraceae families [8]. However, the grandinolide (**4**) was isolated from *T. schomburgkii* [9]. It has been proposed that the biosynthesis of the lactone compounds requires condensation of either a cinnamoyl or benzoyl moiety with a polyketide chain, and subsequent condensation with pyruvic acid [8]. Therefore, the isolation of **3** seems to be good evidence for this biosynthetic proposal. As far as we know this is the first report of ω-phenyl alkanoic acids from plants. However, ω-cyclohexyl and ω-cycloheptyl fatty acids have been isolated from the lipids of the thermoacidophile *Alicyclobacillus acidocaldarius* and *A. cycloheptanycus* [10]. The biosynthesis proposed for these compounds is based exclusively on the polyketide pathway.

EXPERIMENTAL

General. IR: KBr. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$ with TMS as int. standard. GC-MS: low resolution on a HP-2576 instrument.

Isolation of constituents. Leaves of *T. claussenii* were collected in Rio Claro, SP, Brazil, and a voucher is deposited in the Herbarium of the Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP, Brazil. The leaves were dried, powdered and extracted with hexane, CH_2Cl_2 and MeOH. The hexane extract (28.7 g) was submitted to vacuum chromatography over silica gel using hexane, CH_2Cl_2, EtOAc and MeOH. The hexane fr. was chromatographed over silica gel using hexane–EtOAc (19:1), yielding caryophyllene epoxide (14.9 mg). Chromatography of the CH_2Cl_2 fr. over silica gel using hexane–EtOAc (99:1) followed by prep. TLC (hexane–EtOAc, 9:1), yielded plastocromenol (30.0 mg) and a mixt. of terpenoid fatty acid derivatives. This mixt., after hydrolysis with alcoholic KOH and flash chromatography using hexane–EtOAc (9:1), afforded 24-methylenecycloartanol (**2b**) (10.0 mg), and a mixt. of sitosterol and stigmasterol. The fatty acids obtained from hydrolysis were esterified with CH_2N_2 and analysed by GC-MS. The EtOAc fr. was flash chromatographed over silica gel using hexane–EtOAc (9:1) followed by chromatography over florisil using hexane–EtOAc (4:1), yielding 24-methylene-26-hydroxycycloartan-3-one (**1**) (51.0 mg). Compound **1** was acetylated with acetic anhydride and pyridine to yield the monoacetate (**1a**). Chromatography of the last fr. of the EtOAc fr., firstly, over silica gel using hexane–EtOAc (4:1) and then over silica gel (flash) using hexane–Me_2CO (99:1), yielded a mixt. of ω-phenyl alkanoic and alkenoic acids (**3**) (80.0 mg), which was esterified with CH_2N_2 and analysed by GC-MS. From the CH_2Cl_2 extract using a similar procedure as above it was possible to isolate **1**. The MeOH extract (19.9 g) was suspended in MeOH–H_2O (1:3) and partitioned with CH_2Cl_2–EtOAc–*n*-BuOH. The CH_2Cl_2 fr. was concd and then partitioned with hexane–MeOH. The MeOH fr. was chromatographed on a DCCC column ($CHCl_3$–MeOH–H_2O), 5:5:3, ascending method). This led to the isolation of a mixt. of sitosterol and stigmasterol glycosides. The last fr. eluted afforded, after flash chromatography over silica gel using Me_2CO–H_2O (4:1), the amino acids *N*-methylproline (66.4 mg) and 4-hydroxy-*N*methylproline (120.3 mg).

24-Methylene-26-hydroxycycloartan-3-one (**1**) Amor phous solid, mp 138–140°, $[\alpha]_D$ +18.7° ($CHCl_3$, *c* 0.05), EIMS *m/z* (rel. int.): 424 $[M-CH_2O]^+$ (39), 355 (21), 313 (39), 271 (14), 201 (22), 147 (48), 107 (68), 95 (92), 91 (58), 55 (100). ^{13}C NMR ($CDCl_3$): δ 216.8 (*s*, C-3), 152.0 (*s*, C-24), 109.5 (*t*, C-31), 65.8 (*t*, C-26), 52.2 (*d*, C-17), 50.2 (*s*, C-4), 48.7 (*d*, C-5), 48.4 (*s*, C-14), 47.9 (*d*, C-8), 45.3 (*s*, C-13), 42.5 (*d*, C-25), 37.5 (*t*, C-2), 36.1 (*d*, C-20), 35.5 (*t*, C-12), 34.0 (*t*, C-23), 33.4 (*t*, C-1), 32.8 (*t*, C-15), 31.2 (*t*, C-22), 29.5 (*t*, C-19), 28.1 (*t*, C-7), 26.7 (*t*, C-16), 25.9 (*t*, C-11), 25.8 (*s*, C-10), 22.2 (*q*, C-29), 21.5 (*t*, C-6), 21.0 (*s*, C-9), 20.8 (*q*, C-30), 19.3 (*q*, C-28), 18.3 (*q*, C-21), 18.1 (*q*, C-18), 16.4 (*q*, C-27). The multiplicities were obtained from DEPT 135.

24-Methylene-26-acetoxycycloartan-3-one (**1a**). ^{13}C NMR ($CDCl_3$): δ 216.7 (*s*, C-3), 171.2 (*s*, OCOMe), 151.5 (*s*, C-24), 109.0 (*t*, C-31), 68.1 (*t*, C-26), 52.2 (*d*, C-17), 50.2 (*s*, C-4), 48.7 (*d*, C-5), 48.4 (*s*, C-14), 47.9 (*d*, C-8), 45.3 (*s*, C-13), 38.7 (*d*, C-25), 37.5 (*t*, C-2), 36.0 (*d*, C-20), 35.5 (*t*, C-12), 34.7 (*t*, C-23), 33.4 (*t*, C-1), 32.8 (*t*, C-15), 31.6 (*t*, C-22), 29.5 (*t*, C-19), 28.1 (*t*, C-7), 26.7 (*t*, C-16), 25.9 (*s*, C-10), 25.8 (*t*, C-11), 22.1 (*q*, C-29), 21.5 (*t*, C-6), 21.1 (*q*, OCOMe), 21.0 (*s*, C-9), 20.8 (*q*, C-30), 19.3 (*q*, C-28), 18.3 (*q*, C-21), 18.1 (*q*, C-18), 17.0 (*q*, C-27). ^{1}H NMR ($CDCl_3$): δ 4.78 (*sl*, H-31a), 4.75 (*sl*, H-31b), 4.07 (*dd*, J = 6.0, 10.8 Hz, H-26a), 3.91 (*dd*, J = 7.2, 10.8 Hz, H-26b), 2.69 (*td*, J = 6.4; 13.6; 13.6 Hz, H-2β), 2.42 (*sext.*, J = 6.8 Hz, H-25), 2.28 (*ddd*, J = 2.4, 4.0, 13.6 Hz, H-2α), 2.12 (*, H-23a), 1.90 (*, H-23b), 1.85 (*, H-1a), 1.56 (*, H-22a), 1.54 (*, H-1b), 1.38 (*, H-20), 1.25 (*, H-22b), 1.05 (*d*, J = 7.2 Hz, H-27), 0.88 (*d*, J = 6.4 Hz, H-21), 0.76 (*d*, J = 4.4 Hz, H-19a), 0.55 (*d*, J = 4.4 Hz, H-19b). * Multiplicities not determined.

ω-Phenyl-alkanoic and alkenoic methyl esters of (**3**). Oil. ^{13}C NMR ($CDCl_3$): δ 174.6 (*s*), 143.2 (*d*), 143.1 (*d*), 130.2 (*d*), 130.1 (*d*), 128.7 (*d*), 128.5 (*d*), 125.9 (*d*), 125.8 (*d*), 51.7 (*q*), 36.3 (*t*), 34.4 (*t*), 31.8 (*t*), 31.7 (*t*), 29.9 (*t*), 29.8 (*t*), 29.7 (*t*), 29.6 (*t*), 29.5 (*t*), 29.4 (*t*), 29.3 (*t*), 29.2 (*t*), 25.2 (*t*).

GC/MS analysis. Samples (1 μl) were injected (split/splitless) into an HP-1 capillary column (25 m × 0.2 mm i.d., 0.33 μm film thickness) at an oven temp. of 120° and a H flow rate of 1 ml min^{-1}. The oven temp. was increased at 5° min^{-1} to 280°. FID detector temp. was 280°. Peak identities were confirmed by GC–MS. The interface temp. was 280°. Positive-ion EIMS were acquired at 70 eV by scanning from 480-50 mu sec^{-1}.

ω-Phenyl decanoic acid methyl ester. R_t = 10.151 min, EIMS *m/z* (rel. int.): 262 $[M]^+$ (6), 230 (11), 139 (5), 105 (16), 91 (100), 74 (30).

ω-Phenyl undecanoic acid methyl ester. R_t = 20.18 min, EIMS *m/z* (rel. int.): 276 $[M]^+$ (8), 244 (15), 153 (5), 117 (8), 91 (100), 74 (27).

ω-Phenyl dodecanoic acid methyl ester. R_t = 22.41 min, EIMS *m/z* (rel. int.): 290 $[M]^+$ (10), 258 (26), 131 (6), 91 (100), 74 (26).

ω-Phenyl tridecanoic acid methyl ester. R_t = 24.32 min, EIMS *m/z* (rel. int.): 304 $[M]^+$ (11), 272 (30), 131 (8), 91 (100), 74 (26).

ω-Phenyl tetradecanoic acid methyl ester. R_t = 25.82 min, EIMS *m/z* (rel. int.): 318 $[M^+]$ (8), 286 (34), 131 (9), 91 (100), 74 (26).

ω-Phenyl pentadecanoic acid methyl ester. R_t = 27.53 min, EIMS *m/z* (rel. int.): 332 $[M]^+$ (8), 300 (36), 131 (10), 91 (100), 74 (28).

ω-Phenyl tetradecenoic acid methyl ester. R_t = 25.29 min, EIMS *m/z* (rel. int.): 316 $[M]^+$ (9), 284 (9), 131 (19), 104 (100), 91 (84), 55 (25).

ω-Phenyl pentadecenoic acid methyl ester. R_t = 27.05 min, EIMS *m/z* (rel. int.): 330 $[M]^+$ (11), 298 (11), 145 (6), 104 (100), 91 (83).

Acknowledgements—The authors are grateful to Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Financiadora de Estudos e Projetos (FINEP). One of us (M.T.P.) acknowledges Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for the award of a scholarship.

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