Studies on the Constituents of *Commiphora mukul*

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Studies on *ommiphora mukul* (Hook, ex stock) Engl. have led to the isolation of a new lignan (+)-commiphorin (**1**), a new fatty acid ester, (+)-commiphotetrol (**2**) along with (–)-hydroxyisohopane (**3**) [1], which is the first report of this compound from *Commiphora mukul* of Pakistan origin, Z and *E*-guggulsterones, cholesterol and guggulsterol-II (**4**). Of these complete ^{1}H and ^{13}C NMR data of guggulsterol-II (**4**) is assigned for the first time [2]. The structures of the compounds were elucidated with the help of extensive spectroscopic studies [3, 4]. Compound **1** have shown antibacterial activity against a number of bacteria. *Amoxicillin* $(H_2O)_3$, *Ampicilin* and *Cefuroxime* were used as positive control (Table 2).

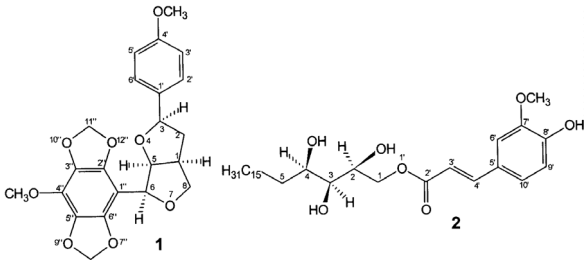
Key words: Commiphora mukul, Fatty Acid Ester, Antibacterial Activity, Lignan

Introduction

Commiphora mukul (Burseraceae) is widely distributed in Pakistan and India and its gum-resin is claimed to be efficacious in the treatment of rheumatism, arthritis and allied disorders [5]. Pharmacological studies on the crude drug, as well as some fractions and pure constituents of *C. mukul* have revealed significant anti-inflammatory, antirheumatic and hypocholestremic/hypolipaemic activities [6, 7]. Previous phytochemical studies on this plant have resulted in the isolation of steroids, terpenoids, glycosides and carbohydrates *etc*. In this communication, we report the isolation and structure elucidation of a new lignan, (+)-commiphorin (**1**) and a new fatty acid ester, (+)-commiphotetrol (**2**) from the ethyl acetate extract of the gum-resin of *C. mukul* of Pakistani origin.

Results and Discussion

(+)-Commiphorin (**1**) was isolated as a colorless amorphous solid from the ethyl acetate extract of the gum-resin of *C. mukul* by column and thin-layer chromatography. The UV spectrum of compound **1** showed absorptions at 276 and 245 nm characteristic for the methylene dioxy-bearing benzene ring [8]. The IR spectrum afforded intense absorptions at 2850 (C-H), 1630 (aromatic C=C), 1136, 1090 (C-O) cm^{-1}. The high resolution electron-impact mass spectrum of (**1**) showed the molecular ion-peak at *m/z* 414.1278 which corresponded to the molecular formula $C_{22}H_{22}O_8$ and indicated the presence of twelve degrees of unsaturation in the molecule. The ion at *m/z* 383 was due to the loss of the *O*-methyl group from the molecular ion.



The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **1** showed 3H singlets at $\delta = 3.90$ and 3.91 due to two methoxy groups substituted at C-4' and C-4". Two 1H doublets at $\delta = 6.53$ ($J_{2',3'} = 1.4$, H-2') and $\delta = 6.50$ ($J_{3',2'} = 1.4$ Hz, H-3') were due to the two aromatic protons. The other two aromatic protons appeared at $\delta = 6.55$ (1H, d, $J_{5',6'} = 1.4$ Hz, H-5') and 6.57 (1H, d, $J_{6',5'} = 1.2$ Hz, H-6'). Three 1H doublets at $\delta =$ 4.38 ($J_{6,5} = 7$ Hz, C-6), 4.81 ($J_{3,2} = 5.3$ Hz, C-3) and 3.25 ($J_{5,6} = 2$ Hz, C-5) were also observed. The one-bond ^{13}C/^{1}H couplings were determined by the HMQC spectrum (Table 1), while the vicinal and long-range ^{1}H/^{1}H couplings were established on the basis of COSY-45° and HOHAHA (20, 60, 100 ms) experi-

Table 1. ^{13}C and ^{1}H NMR spectral data of **1**, **2** and **4**.

	1			**2**			**4**		
Compl. No.	^{13}C NMR δ (ppm)	m*	^{1}H NMR# δ (ppm) *J* (Hz)	^{13}C NMR δ (ppm)	m*	^{1}H NMR δ (ppm) *J* (Hz)	^{13}C NMR δ (ppm)	m*	^{1}H NMR δ (ppm) *J* (Hz)
1	54.6	CH	2.84, m	66.0	CH_2	4.37, m	23.8	CH_2	1.9* and 2.0*, m
2	69.6	CH_2	3.27* and 3.33* m	73.4	CH_2	3.76, m	32.3	CH_2	1.75* and 1.76*, m
3	82.0	CH	4.81, d (5.3)	72.3	CH_2	4.02, m	72.4	CH_2	3.38, m
4	–	–	–	72.1	CH_2	3.46, q	43.0	CH_2	2.19* and 2.22*, m
5	50.0	CH	3.25, d (2)	33.7	CH_2	1.57, m	142.2	–	–
6	87.6	CH	4.38, d (7)	31.9	CH_2	1.26, m	122.2	CH_2	5.32, d (4.8)
7				29.6	CH_2	1.24, m	32.8	CH_2	1.3* and 11.4*, m
8	71.0	CH_2	3.88* and 4.11*, m	29.6	CH_2	1.24, m	32.3	CH_2	1.57, m
9				29.6	CH_2	1.24, m	51.7	CH_2	0.9, m
10				29.6	CH_2	1.24, m	37.7	–	–
11				29.6	CH_2	1.24, m	21.8	CH_2	1.53* and 1.54*, m
12				29.6	CH_2	1.24, m	40.9	CH_2	1.24* and 11.28*, m
13				29.6	CH_2	1.24, m	43.9	–	-
14				29.6	CH_2	1.24, m	55.9	CH	0.85, m
15				29.6	CH_2	1.24, m	38.4	CH_2	1.86* and 1.88*, m
16				29.6	CH_2	1.24, m	74.4	CH_2	4.58, m
17				29.6	CH_2	1.24, m	61.0	CH_2	1.20, m
18				25.5	CH_2	1.24, m	15.2	CH_3	1.13, s
19				22.6	CH_2	1.24, m	19.8	CH_3	1.02, s
20				14.0	CH_3	0.86, t	78.1	–	–
21							26.5	CH_3	1.25, s
22							45.4	CH_2	1.58*, 1.76*, s
23							41.6	CH_2	2.11* and 2.14*,m
24							38.1	CH_2	1.29* and 1.31*,m
25							29.0	CH_2	1.57, m
26							22.9*	CH_3	0.90, s
27							23.1	CH_3	0.88, s
1’	133.0	–							
2’	105.7	CH	6.53, d (1.4)	168.0	–	–			
3’	99.9	CH	6.50, d (1.4)	114.6	CH	6.29, d (16)			
4’	143.5	–	–	146.0	CH	7.65, d (16)			
5’	100.3	CH	6.55, d (1.4)	126.8	–	–			
6’	105.1	CH	6.57, d (1.2)	109.5	CH	7.01, d (1.6)			
7’	–			146.8	–	–			
8’	–			148.3	–	–			
9’	–			114.8	CH	6.90, d (4.0)			
10’				123.3	CH	7.06, dd (1.6, 6.8)			
1”	135.9								
2”	134.8								
3”	148.9								
4”	143.7								
5”	149.1								
6”	134.2								
8”	101.4	CH_2	5.94						
11”	101.3	CH_2	5.95						
-OCH_3	56.0	CH_3	3.90* and 3.91*, s	56.0	–	3.90, s			

* Multiplicity; # one-bond heteronuclear correlations determined by HMQC experiment.

ments. The C-2’ aromatic proton ($\delta = 6.53$) was found to be coupled with the C-3’ aromatic proton ($\delta = 6.50$) in the COSY-45° spectrum. Similarly the C-5’ aromatic proton ($\delta = 6.55$) also showed a cross-peak with the C-6’ aromatic ring proton ($\delta = 6.57$). A 1H multiplet at $\delta = 2.84$ (C-1), showed ^{1}H-^{1}H shift correlations with the C-2 methylene ($\delta = 3.27$ and 3.33) and C-8 methylene ($\delta = 3.88$, 4.11) protons as well as with the C-5 methine proton ($\delta = 3.25$). The C-5 methine proton ($\delta = 3.25$) showed a cross-peak with the C-6 methine proton ($\delta = 4.38$), while the C-3 methine proton ($\delta = 4.81$) interacted with the C-2 methylene protons ($\delta = 3.27$ and 3.33). The downfield shift of the C-3 methine proton indicated the presence of geminal oxygen

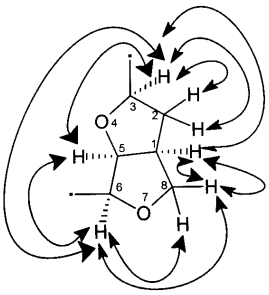


Fig. 1a. HOHAHA interactions for compound **1**.

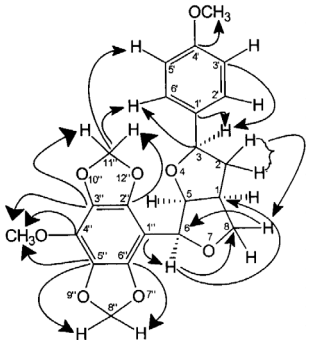


Fig. 1b. Selected HMBC interactions for compound **1**.

functionality. The C-6 methine proton similarly exhibited COSY-45° interactions with the C-5 methine proton, and its downfield chemical shift also reflected the presence of geminal oxygen and benzene functionalities. The C-3 methine proton ($\delta = 4.81$) thus interacted with the H-1 ($\delta = 2.84$), H-5 ($\delta = 3.25$), H-6 ($\delta = 4.38$) and H-8 ($\delta = 3.88$ and 4.11). The HOHAHA interactions of protonated carbons are shown in Fig 1a.

The ^{13}C NMR broad-band spectrum ($CDCl_3$, 100 MHz) of **1** showed the resonances of 21 carbon atoms in the molecule (two carbons, $\delta = 56.0$) have overlapping chemical shift. The DEPT spectra revealed the presence of eight methine, four methylene and two methyl carbon atoms and by the difference of DEPT spectra from the broad-band spectrum, the presence of eight quaternary carbon atoms. The C-3 ($\delta = 82.07$) and C-6 ($\delta = 87.66$) carbons downfield chemical shifts reflecting the presence of geminal oxygen and benzene moieties. Complete ^{13}C NMR chemical shift assignments of compound **1** are shown in Table 1. Complete ^{1}H/^{13}C one-bond shift correlations of protonated carbon atom are presented in Table 1. In the HMBC spectrum, the methylene protons at $\delta =$ 5.94 (H-8") and 5.95 (H-11") showed long-range shift correlations with the quaternary carbon atoms resonating at $\delta = 134.8$ (C-2"), 134.2 (C-6"), 148.9 (C-3") and 149.1 (C-5"). The protons of the methoxy group exhibited interactions with the quaternary carbons at $\delta = 148.9$ (C-3"), 143.7 (C-4"), 149.1 (C-5") and 143.6 (C-4'). The other important HMBC interactions are shown around Fig. 1b. These studies led to structure **1** for the compound. Commiphorin (**1**), showed good antibacterial activity against *Staphylococcus aureus*, *Streptococus pyogenes*, *Corynebacterium diphtheriae* and *Escherichia coli*. *Amoxicillin*(H_2O), *Ampicillin* and *Cefuroxine* serving as a positive control (Table 2).

Table 2. Antibacterial activity of (+) commiphorin (**1**).

Bacteria	Zone of inhibition (Sample 1) (200 μg/ml)	Zone of inhibition (Ref. Drug) (100 μg/ml)
Staphylococus aureus	6	17[a], 17[b], 17[c]
Streptococous pyogenes	6	19[a], 18[b], 18[c]
Corynebacterium diphtheriae	7	12[a], 12[b], 13[c]
Escherichia coli	7	19[b], 18[a], 18[c]

[a] Amoxicillin $(H_2O)_3$; [b] ampicillin; [c] cefuroxine.

(+)-Commiphotetrol **2** showed UV absorption at 325 nm, consistent with the presence of a conjugated benzene chromophore. Its IR spectrum exhibited absorption bands at 3520 (OH), 2845, 2912 (C-H), 1700 (C=O), 1630, 1592 (C=C) and 1160 (C-O) cm^{-1}. The HREI MS of the compound exhibited the molecular ion at *m/z* 522.3580 corresponding to the molecular formula $C_{30}H_{50}O_7$ (calcd. 522.3556) indicating the presence of six degrees of unsaturation in the molecule. This suggested that the compound has six double bond equivalents in the molecule.

The ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of **2** showed three aromatic 1H signals at $\delta = 7.01$ (d, $J_{6',10'} = 1.6$ Hz, H-6'), 7.06 (dd, $J_{10',6'} = 1.6$ Hz, $J_{10',9'} = 6.8$ Hz, H-10') and $\delta = 6.90$ (d, $J_{9',10'} =$ 4.0 Hz, H-9'). Three 1H signals at $\delta = 3.46$ (t, H-4), 4.02 (m, H-3) and 3.76 (m, H-2) corresponded to three secondary hydroxyl groups. A 3H singlet at $\delta = 3.91$ indicated the presence of a methoxy group. Two 1H doublets at $\delta = 6.29$ ($J_{3',4'} = 16$ Hz, H-3') and $\delta =$ 7.65 ($J_{4',3'} = 16$ Hz, H-4') were assigned to the α,β-olefinic protons. These data suggested the presence of ferulic acid unit in the molecule due to the close matching of the data with the literature values [9]. A 3H triplet resonated at $\delta = 0.86$, two 2H multiplets resonated at $\delta = 1.57$ (H-5) and 4.37 (H-1), while a broad intense peak resonated at $\delta = 1.23-1.25$, integrating for twenty six protons, (H-7 to H-19) indicating the prescence of an aliphatic methylene chain

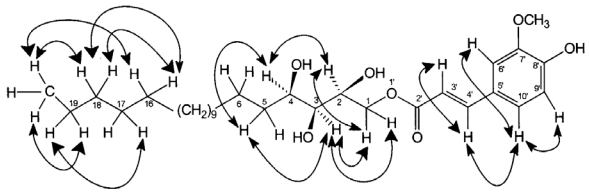


Fig. 2a. HOHAHA interactions for compound **2**.

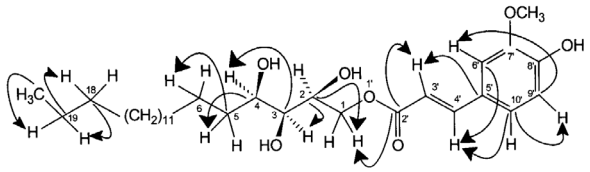


Fig. 2b. Selected HMBC interactions for compound **2**.

in the molecule. In the COSY-45° spectrum, methine H-10' ($\delta = 7.06$) showed cross-peak with the C-9' methine proton ($\delta = 6.90$). H-3' ($\delta = 6.30$) was coupled with H-4' ($\delta = 7.65$), while H-1 ($\delta = 4.37$) was found to be coupled with H-2 ($\delta = 3.76$). The latter also exhibited coupling interaction with H-3 ($\delta = 4.02$). H-4 also showed a cross-peak with H-5 ($\delta = 1.57$) which in turn showed vicinal couplings with H-6 ($\delta =$ 1.26 and 1.27). Cross-peaks were also observed between H-18 ($\delta = 1.25$) and H-19 ($\delta = 1.30$). The latter showed coupling with H-20 ($\delta = 0.86$) in the COSY-45° spectrum. The HOHAHA interactions are presented in Fig. 2a.

The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of (**2**) showed twenty one resonances (since nine carbons C-7, 8, 9, 10, 11, 12, 13, 14 and 15 resonated at the same chemical shift value due to the similar environment). The DEPT spectra recorded at different pulse angles ($\theta = 45°$, 90° and 135°) revealed the presence of two methyl, eight methylene and eight methine carbon atoms in the molecule. By the difference DEPT signals from the broad-band spectrum, it was concluded that there were four quaternary carbon atoms. The chemical shift values are presented in Table 1. Complete ^{1}H/^{13}C one-bond shift correlations of every protonated carbon atom is presented in Table 1. The long-range heteronuclear interactions i.e. HMBC spectrum exhibited couplings between the methine proton resonating at $\delta = 7.65$ with the quaternary carbon at $\delta = 126.8$ (C-5'), C-2' ($\delta = 168.00$) and C-10' ($\delta =$ 123.3). H-1 ($\delta = 4.37$) also showed shift correlation with the quaternary C-2' ($\delta = 168.00$). The various HMBC interactions are shown in Fig. 2b. These studies led to structure **2** for the compound.

Experimental Section

General experimental procedure- The ^{1}H NMR spectra were recorded on a Bruker AM-400 instrument at 400 MHz, while the ^{13}C NMR spectra were recorded on the same instruments at 100 or 125 MHz, respectively. The mass spectra were recorded on the same instruments at 125 MHz, respectively. The mass spectra were recorded on a Varian MAT 312 S double focusing mass spectrometer. HREI MS were recorded on a Jeol-JMS HX 110 mass spectrometer. CC was performed on silica-gel (type 60, 70-230 mesh, E. Merck). TLC experiments were carried out on silica gel precoated plates (E. Merck, 0.25 mm).

Extraction and isolation-"Guggulu" gum-resin (150 gm) was dissolved in methanol (1.0 l) and defatted with pet.ether (3 l). The defatted MeOH extract was evaporated to yield a crude gum (120 gm), which was loaded onto a silica gel column (Merck, 70-230 mesh, 3600 gm) and eluted with gradients of mixtures of pet. ether, chloroform, ethyl acetate and methanol. Compound **1** was isolated from fractions 20 – 30 (19.82 mg) (500 ml each) obtained with pet. ether: EtOAc (8 : 2) eluates using the same solvent system and washing with methanol, obtained the pure compound 1, (17 mg, 0.014% yield). Successive chromatography was achieved by using increasing amount of EtOAc in pet. ether.

(+)-Commiphotetrol (**2**) was obtained from fractions 70 – 82 (33.82 mg) 500 ml each as a colorless amorphous solid on elution with chloroform-methanol (9 : 1) and subjected to preparative TLC using chloroform-methanol (6 : 4) followed by washing with methanol to afford pure **2** (30 mg, 0.025% yield). (–)-Hydroxyisohapanone (**3**) was isolated from fractions 31 – 45 (28.92 mg) 500 ml each obtained on elution of the above column with pet. ether : ethyl acetate (8 : 2), after repeated CC and washing with methanol to afford (**3**) (25.10 mg, 0.03% yield). Guggulsterol-II (**4**) was isolated from frations 85 – 98 (509.72 mg) 500 ml each obtained on elution of the above column with pet. ether : ethyl acetate (6 : 4) and washing with methanol to afford (**4**) (500 mg, 0.4% yield).

Antibacterial bioassay [10]

Antibacterial bioassay was determined by agar well diffusion method. This test was performed by spreading 18 – 24 h old pathogenic bacterial cultures containing approximately $10^4 - 10^6$ colony forming units (CFU/ml) on the surface of nutrient agar (Bio M Laboratories, USA BMO 13 – 62 N) plates. Wells were dugged in the media with the help of a sterile metallic borer. Test samples of different concentrations prepared in dimethyl sulfoxide (DMSO, Merck) are added in their respective wells. Pure DMSO was used as a control. Other wells are supplemented with reference. Antibiotics *i.e. amoxycillin*.$3H_2O$, *ampicillin*.$3H_2O$, *tetracycline* and *cefuroxin* –Na^+ serving as positive control [10].

(+)-Commiphorin (**1**): Colorless amorphous solid; $[\alpha]_D^{254}$ ($CHCl_3$) (+)97.3° ($c = 0.1\%$); IR ($CHCl_3$)ν_{max}: 2850 (C-H), 1630 (aromatic C=C), 1136, 1090 (C-O) cm^{-1}; UV ($CHCl_3$)λ_{max}: 245 and 276 nm ($\log\varepsilon$): 3.73. For ^1H and ^{13}C NMR see Table 1. HREI MS:*m/z* (rel. int. %): 414.1278, $C_{22}H_{22}O_8$ (100%); EIMS: *m/z* (rel. int %) M^{+1} 414 (100), [M-OCH_3]$^+$ 383 (2), [M-$C_{10}H_{13}O_3$]$^+$ 233 (18), [M-$C_{10}H_{12}O_4$]$^+$ 218 (8), [M-$C_{11}H_{10}O_4$]$^+$ 208 (30), [M-$C_{13}H_{15}O_4$]$^+$ 179 (74), [M-$C_{13}H_{13}O_5$]$^+$ 165 (70), *M-$C_{13}H_{15}O_4$* 179 (75), [M-$C_{14}H_{14}O_5$]$^+$ 152 (24), *M-$C_{14}H_{17}O_6$]$^+$ 133 (5), [M-$C_{18}H_{15}O_8$]$^+$ 56 (15).

(+)-Commiphotetrol (**2**): Colorless amorphous form. $[\alpha]_D^{254}$ (+)17.85° ($CHCl_3$) ($c = 0.11\%$); IR ($CHCl_3$)ν_{max}: 3520 (OH), 2845, 2912 (C-H), 1700 (C=O), 1630, 1592 (C=C) and 1160 (C-O) cm^{-1}; UV (MeOH)λ_{max}: 325 nm ($log\varepsilon$): 3.49. For ^1H NMR and ^{13}C NMR see Table 1. HREI MS: *m/z* (rel.int. %): 522.3580 (25.67%), $C_{30}H_{50}O_7$; EIMS: *m/z* (rel.int %) M^{+1} 522 (3), [M-CH_2CH_3]$^+$ 494.3 (4), [M-$C_{17}H_{35}O$]$^+$ 267.1 (3), [M-$C_{18}H_{38}O_2$]$^+$ 236 (4), [M-$C_{20}H_{40}O_3$] 194.1 (82), [M-$C_{20}H_{41}O_4$] 177.1 (100), [M-$C_{21}H_{45}O_5$]$^+$ 145 (5), [M-$C_{27}H_{44}O_5$]$^+$ 74.1 (14).

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