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Synthesis and Anti-microbial studies of (*E*)-4-Oxoalk-2-enoic acids

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ABSTRACT

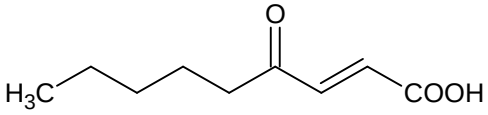
*(E)-4-Oxonon-2-enoic acid **1a** is a fatty acid, isolated from the mycelium of Streptomyces olevaceus. Owing to its significant activity against various Gram-positive and Gram-negative strains and particularly Staphylococcus aureus ATCC 11632; it is an attractive target for structure-activity relationship. We report herein a straightforward synthesis of (E)-4-Oxonon-2-enoic acid **1a** and its analogues in a single step using Wittig reaction. The antibacterial and antifungal activity assay of **1a** and its analogues **1b-1g** is presented.*

Keywords: (*E*)-4-Oxonon-2-enoic acid, Synthesis, Phosphorane, Wittig reaction, Anti-microbial activity.

INTRODUCTION

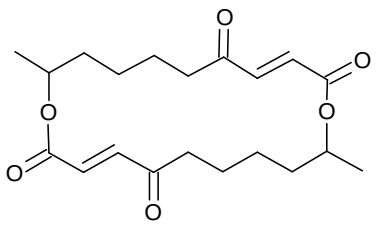
In current chemical and medicinal research, synthesis and biological evaluation of a potentially new antibiotic agent is important. Due to multi-resistance being developed by several microbial strains against commonly used pharmaceutical drugs, there is demand for designing more effective antibiotics with minimum unwanted side effects.

(*E*)-4-Oxonon-2-enoic acid **1a** was isolated from the mycelium of *Streptomyces olevaceus* Tu 4018 by Pfefferle *et al* [1]. Compound **1a** is important for its antibacterial activity against various Gram-positive and Gram-negative strains, especially *Staphylococcus aureus* ATCC 11632 [1].

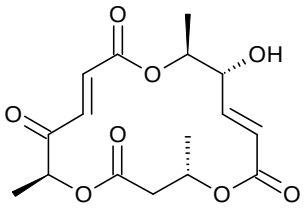


1a

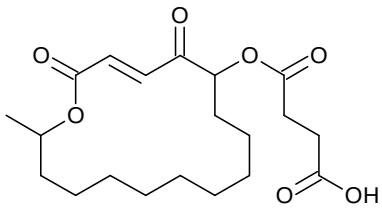
4-oxo-2-enoic acids and their derivatives in general display good biological activity. For example, (*E*)-4-phenyl-4-oxobut-2-enoic acid and its derivatives act as inhibitors of Kynurenine-3-hydroxylase (KYN-3-OHase), an enzyme involved in the metabolism of kynurenine [2] and hence may be useful in the prevention and/or treatment of neurodegenerative diseases.



(±)-Pyrenophorin **2**



Macrosphelide B **3**



(±)-A26771B **4**

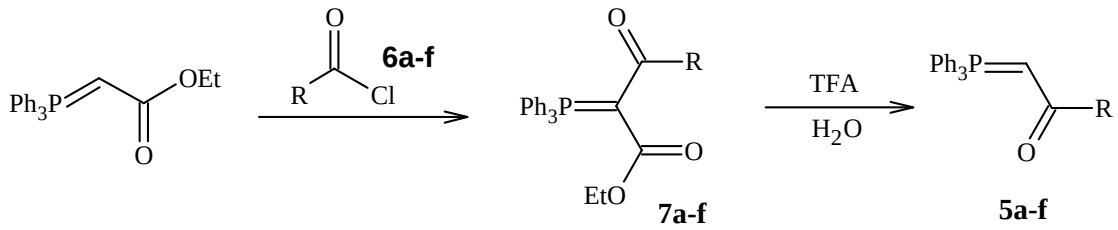
The amides of such acids are cytoprotective and promote the healing of stomach ulcers [3]. The 4-oxo-2-enoic acid unit is also present in many biologically active natural products, such as pyrenophorin **2** [4,5], Macrosphelide B **3** [6] and A26771B **4** [7,8]. Acylacrylic subunit appears to have broad potential as crystallization induced asymmetric transformation (CIAT) template [9].

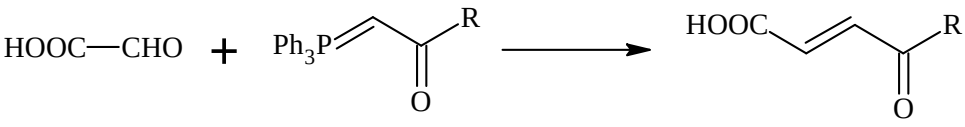
The promising activity of **1a** in anti-bacterial assays and its being a natural product [1] made it a good synthetic target [10,11]. Most of the reported methods for such compounds are either lengthy or costly and specific. In view of this we have developed a general synthesis protocol to make (*E*)-4-Oxonon-2-enoic acid **1a** and its analogues. Further Anti-bacterial and anti-fungal assays of these analogues were carried out.

EXPERIMENTAL SECTION

The melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded on a Shimadzu FTIR spectrophotometer (KBr pellet). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, on a 300 MHz Brucker instrument. The multiplicities of carbon signals were obtained from Distortionless Enhancement by Polarization Transfer (DEPT) experiment. Chemical shifts (ppm) are relative to the internal standard Me_4Si (0 ppm). Thin layer chromatography was performed on silica gel-G. Glyoxalic acid 50% solution was purchased from Aldrich chemical company.

Initially we investigated the synthesis of 4-oxonon-2-enoic acid **1a** a known natural product. Thus Wittig reaction of phosphorane **5a** with glyoxalic acid was carried out at room temperature in chloroform-methanol mixture (Scheme I). As anticipated **1a** was obtained in 67% yield. For synthesis of analogues of **1a**, the corresponding phosphorane **5b-f** were required. These were prepared by acylating ethyl(triphenylphosphoranylidene) acetate using acid chlorides **6b-f** followed by decarboxylative hydrolysis of the keto-ester-phosphorane **7b-f** obtained (Scheme I). The keto-phosphorane **5b-g** on condensation with glyoxalic acid under the same conditions used for preparing **1a** gave analogous acids **1b-g.**





Here R = a) Pentyl, b) Ethyl, c) Propyl, d) n-Butyl, e) Isobutyl, f) Heptyl, g) Phenyl

Scheme I

The phophorane **5g** having a benzene ring was prepared by condensation [12] of α-bromoacetophenone with triphenyl phosphine in dry benzene.

General Procedure for the Preparation of Keto-phosphorane (5):

Thionyl chloride (1.2 equivalents) was slowly added to the cooled acid (1 equivalent) and refluxed it for 4.0 h. Excess of thionyl chloride was then removed by distillation. The residual crude acid chlorides **6a-f** were purified by distilling under vacuum to obtain light yellow liquids and were used for the next reaction.

Ethyl(triphenylphosphoranylidene) acetate (2 equivalent) was added to the solution of freshly prepared acid chlorides (1 equivalent) in 15 mL of toluene. The solid obtained after stirring for 5.0 h at room temperature, was filtered. The keto-ester-phosphoranes **7a-f** obtained after concentration of the filtrate under reduced pressure was in 82 – 96% yield with respect to the acid chlorides.

A solution containing keto-ester-phosphorane **7a-f** (10 mmol) in a mixture of trifluoroacetic acid (15 mL) and water (5 mL) was heated under reflux for 6.0 h. The reaction mixture was then poured onto ice and basified with 2% sodium bicarbonate solution. This was followed by extraction with ethyl acetate (3 X 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using 20% ethyl acetate in petroleum ether to obtain 50 – 74% of the targeted keto-phosphorane **5a-f**. The percentage yield and melting points are given in Table I.

Table I: Yields and Melting points of the Keto-phosphoranes 5

Compounds	R	Yield[a]	m.p.
5a	Pentyl	50%	light yellow oil
5b	Ethyl	74%	215-216°C (lit.[13] 215-218°C)
5c	Propyl	71%	144-145°C (lit.[13] 144-145°C)
5d	Butyl	69%	108-110°C (lit.[14] 109-111°C)
5e	Isobutyl	67%	120-122°C (lit.[14] 120-121°C)
5f	Heptyl	73%	light yellow oil

[a] Isolated yield calculated from **7**.

General Procedure for Preparation of (*E*)-4-oxoalk-2-enoic acid (1a – 1g):
To the solution of keto-phophorane **5a-g (**5 mmol) in 20 mL mixture of chloroform: methanol (1:1), 7.5 mmol of glyoxalic acid was added dropwise while stirring it vigorously. The reaction mixture was stirred at room temperature until the disappearrence of starting material (monitored by TLC). The solvent was removed under vacuum and the solid obtained was dissolved in benzene. Benzene layer was washed well with saturated $NaHCO_3$ solution (7 X 15 mL). The basic aqueous layer was then acidified using dilute HCl to pH 2-3 and the product was extracted in chloroform (4 X 15 mL). All the organic extracts were combined, dried over anhydrous Na_2SO_4, filtered and concentrated to obtain crude acids which were then recrytallized using ethyl acetate-petroleum ether mixture to obtain the products in 59-68% yield of final acids **1a-g**.

Anti-microbial Activity
The antimicrobial activity of the compound was assessed against ten bacterial strains that include Gram-positive bacteria viz *Staphylococcus citreus*, *Methicillin resistant Staphylococcus aureus* (MRSA), *Bacillus subtilis* (MTCC 121), *Staphylococcus* ATCC 25923 and Gram-negative bacteria viz *Salmonella typhimurium, Salmonella paratyphi* A, *Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli* (ATCC 25922), , *Escherichia coli* K-12 (MTCC 1302), also four fungal srains viz *Candida albicans*, *Aspergillus niger, Penicillium* sp. and *Saccharomyces cerevisiae.* Bacterial strains were grown at 37^{o}C for 18 h in shaken flask in nutrient broth (100 mL) and fungal cultures in potato dextrose broth at room temperature (28 ± 2^{o}C)

Conventional disc diffusion method [15] was employed for the assessment of the antimicrobial potential of the compounds. Sterile 6.0 mm diameter blank discs (Whatman No 1 filter paper) were impregnated with the test substances at a compounds concentration of 15 µg/disc. These discs, along with the control disc [(*E*)-4-oxonon-2-enoic acid **1a** a natural antibiotic [1], 15 µg/disc] were placed on petridishes of nutrient agar (for bacterial strains and PDA for fungi) seeded with 0.1 mL of the respective culture broth with the test organism using a sterile pipette and spread plated to obtain a uniform lawn. The plates were incubated at 28 ± 2^{o}C for 24 h. The anti-microbial activity of the compound was determined by measuring the diameter of the zone of inhibition in millimeters.

RESULTS

All the acids displayed carbonyl bands in IR spectrum (KBr if solids and neat if liquids) at around 1720 cm^{-1} and at 1660 cm^{-1}, Also a broad band from 2500 to 3100 cm^{-1}, confirming the presence of -COOH group. The structure and % yield of final products **1a–1g** are given in Table II.

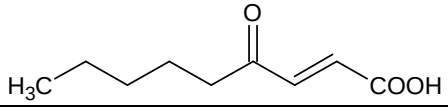
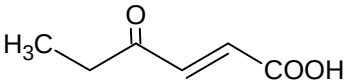
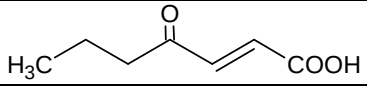
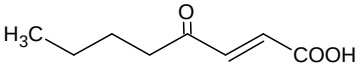
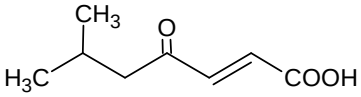
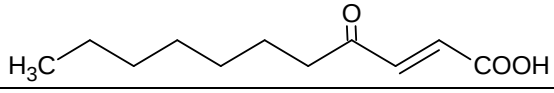
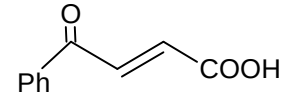
Spectral Details
1a, (*E*)-4-Oxonon-2-enoic acid: ^{1}H NMR ($CDCl_3$, 300 MHz): 0.85 (t, 3H, J = 7.1 Hz); 1.26 (m, 4H); 1.62 (m, 2H); 2.60 (t, 2H, J = 7.2 Hz); 6.62 (d, 1H, J = 15.9 Hz); 7.19 (d, 1H, J = 15.9 Hz), ^{13}C NMR ($CDCl_3$): 13.8 (CH_3); 22.3 (CH_2); 23.2 (CH_2); 31.2 (CH_2); 41.6 (CH_2); 129.4 (CH); 140.9 (CH); 169.0 (Cq); 199.6 (Cq), HRMS: *m/z* calculated for $C_9H_{14}O_3Na$ [(M + Na)$^+$] = 193.0841 found = 193.0844.

1b, **(*E*)-4-Oxohex-2-enoic acid:** ^{1}H NMR ($CDCl_3$ 300 MHz): 1.17 (t, 3H J = 7.2 Hz); 2.7 (q, 2H, J = 7.2 Hz); 6.7 (d, 1H, J = 15.9 Hz); 7.16 (d, 1H, J=15.9 Hz). ^{13}C NMR ($CDCl_3$): 7.5 (CH_3); 35.0 (CH_2); 129.4 (CH); 140.9 (CH); 170.3 (Cq); 199.8 (Cq). GC-MS: M^+ peak at 129.

1c, **(*E*)-4-Oxohept-2-enoic acid:** ^{1}H NMR ($CDCl_3$ 300 MHz): 0.9 (t, 3H, J = 7.2 Hz); 1.7 (m, 2H); 2.65 (t, 2H, J = 7.2 Hz); 6.7 (d, 1H, J = 15.9 Hz); 7.15 (d, 1H, J = 15.9 Hz) ^{13}C NMR ($CDCl_3$): 13.5 (CH_3); 17.1 (CH_2); 43.5 (CH_2); 129.4 (CH); 141.1 (CH); 170.3 (Cq); 199.4 (Cq). GC-MS: M^+ peak at 143.

1d, **(*E*)-4-Oxooct-2-enoic acid:** ^{1}H NMR ($CDCl_3$ 300 MHz): 0.95 (t, 3H, J = 7.5 Hz); 1.38 (m, 2H); 1.65 (m, 2H); 2.67 (t, 2H, J = 7.2 Hz); 6.7 (d, 1H, J = 15.9 Hz); 7.15 (d, 1H, J=15.9 Hz), ^{13}C NMR ($CDCl_3$): 13.7 (CH_3); 22.1 (CH_2); 25.6 (CH_2); 41.4 (CH_2); 129.6 (CH); 141.0 (CH); 170.4 (Cq); 199.7 (Cq), HRMS: *m/z* calculated for $C_8H_{13}O_3$ [$(M + H)^+$] = 157.0864, found = 157.0858.

Table II: Structures and % yields of Final Products 1a – 1g

Products	Structures	% Yields[a]	m.p.
1a		67%	104-105°C (lit. [16]: 105-106°C)
1b		68%	105-106 °C, (lit. [17]: 105-108°C)
1c		61%	108-109 °C (lit. [9]: 107-109°C)
1d		60%	111-112 °C (lit. [17]: 98-100°C)
1e		59%	98-99 °C (lit. [16]: 91.5-92.5°C)
1f		60%	light yellow oil
1g		66%	95-96 °C (lit. [18]: 94-96°C)

[a] Isolated yield calculated from **5**

1e, **(*E*)-6-Methyl-4-oxohept-2-enoic acid:** ^{1}H NMR ($CDCl_3$, 300 MHz): 0.95 (d, 6H, J = 6.6 Hz); 2.2 (m, 1H); 2.52 (d, 2H, J = 6.9 Hz); 6.65 (d, 1H, J = 15.9 Hz); 7.13 (d, 1H, J = 15.9 Hz), ^{13}C NMR ($CDCl_3$): 22.4 (CH_3); 24.7 (CH); 50.5 (CH_2); 129.5 (CH); 141.3 (CH); 170.4 (Cq); 199.3 (Cq), HRMS: *m/z* calculated for $C_8H_{13}O_3$ [$(M + H)^+$] = 157.0864, found =157.0860.

1f, (*E*)-4-Oxoundec-2-enoic acid: ^{1}H NMR ($CDCl_3$ 400 MHz): 0.88 (m, 5H); 1.28 (m, 4H); 1.56 (m, 2H); 1.68 (m, 2H); 2.66 (m, 2H); 6.73 (d, 1H, *J* = 15.9 Hz); 7.23 (d, 1H, *J* = 15.9 Hz), ^{13}C NMR ($CDCl_3$): 11.6 (CH_3); 13.8 (CH_2); 22.7 (CH_2); 24.2 (CH_2); 29.5 (CH_2); 30.5 (CH_2); 52.8 (CH_2); 129.7 (CH); 140.4 (CH); 170.4 (Cq); 202.92 (Cq).

1g, (*E*)-4-Phenyl-4-oxobut-2-enoic acid: ^{1}H NMR ($CDCl_3$ 300 MHz): 6.9 (d, 1H, J = 15.6 Hz); 7.55 (t, 2H, J = 7.5 Hz); 7.66 (t, 1H, J = 7.2 Hz); 8.02 (m, 1H), ^{13}C NMR ($CDCl_3$): 128.89 (CH); 128.94 (CH); 131.39 (CH) 134 (C); 136.3 (C); 138.4 (CH); 170.3 (C); 189.2 (C), HRMS: *m/z* calculated for $C_{10}H_8O_3Na$ [$(M + Na)^+$] = 199.0379, found =199.0372.

Anti-microbial Activity

The results obtained in Antimicrobial Activity Test are summarized in Table III.

Table III: a: Anti-bacterial activity by disc diffusion assay (Inhibition zones in mm)

Bacterial strains	1a	1b	1c	1d	1e	1f	1g
S. citreus	-	8	-	16	11	-	17
S. aureus MRSA	15	-	-	7	12	-	12
B. subtilis	-	-	-	-	-	-	-
Staphylococcus ATCC 25923	10	-	-	-	-	19	10
S. typhimurium	7	-	-	10	9	14	7
S. paratyphi A	11	-	-	10	-	-	12
P. mirabilis	7	-	-	10	-	-	8
K. pneumoniae	-	-	-	-	-	-	-
E. coli ATCC 25922	-	-	-	-	-	16	-
E. coli K-12	19	11	-	10	14	15	18

Table III: b: Anti-fungal activity by disc diffusion assay (Inhibition zones in mm)

Fungal strains	1a	1b	1c	1d	1e	1f	1g
A. niger	16	-	-	10	-	-	14
Penicillium sp.	11	-	-	11	-	-	13
S. cerevisiae	-	-	-	-	-	-	-
C. albicans	18	9	-	13	13	-	-

DISCUSSIONS

A new, convenient and one step synthesis of (*E*)-4-oxoalkyl-2-enoic acids, which includes a natural product **1a**, has been developed. The method is very simple and highly effective. Final acids were characterized based on IR, ^{1}H NMR, ^{13}C NMR and mass spectra.

The Antimicrobial activity of analogues was compared with the natural antibiotic **1a**. The assay showed that analogues **1b, 1c** (R = ethyl, propyl) with smaller alkyl groups were either active against very few microbes or not active at all. Whereas medium alkyl group derivative **1d** (R = butyl) was as active as that of lead compound **1a** (R = pentyl). Its inhibition zones were smaller then the lead compound **1a** but was active against almost all the microbes. The results were interesting in the case of higher derivative (**1f,** R= heptyl) of acid. It exhibited a good inhibition zone but selectively against four microbes including *E. coli* ATCC 25922 where **1a** did not show any activity. The branched alkyl derivative **1e** (R = isobutyl) showed activity against very few

microbes viz *Staphylococuus citreus*, *Methicillin Resistant Staphylococcus aureus* (MRSA), *Salmonella typhimurium* and *Escherichia coli* K-12 (MTCC 1302) compared to its n-alkyl isomer as well as the lead compound **1a**. The aromatic compound **1g** was active against most of the microbes with higher inhibition zone than the lead compound **1a**. Derivatives **1b, 1d, 1e** and **1g** showed remarkable activity against *S. citreus* where even the natural acid **1a** did not exhibit any activity.

Acknowledgement
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REFERENCES

[1] C Pfefferle; C Kempter; J W Metzger; H Fiedler. *J. Antibiotics*, **1996,** 49(8), 826 - 828.
[2] A Giordani; P Pevarello. US Patent US 6048896, 2000.
[3] T Fodor; L Dobay; J Fischer; H Stefkoo; E Ezer; J Matuz; K Sághy; L Szporny. US Patent US 4923888, 1990.
[4] M Petrini; R Ballini; G Rosini; E Marotta. *Tetrahedron*, **1986,** 42(1), 151 – 154.
[5] P Bonete; C Najera. *J. Org. Chem*, **1994,** 59(11), 3202 – 3209.
[6] M Yuji; T Kawaguchi; H Nemoto. *Org. Lett.*, **2003,** 5(16), 2939 - 2941.
[7] M Asaoka; N Yanagida; H Takei. *Tetrahedron Lett*, **1980,** 21(48), 4611 - 4614.
[8] M Kawashima; T Fujisawa. *Chem. Lett*, **1984,** 13(10), 1851 – 1854.
[9] P Jakubec; D Berkes; A Kolarovic; F Povazanec. *Synthesis*, **2006,** 23, 4032 - 4040.
[10] R Ballini; G Bosica. *J. Nat. Prod.*, **1998,** 61(5), 673 - 674.
[11] D Obrect; B Weiss. *Helv. Chim. Acta*, **1989,** 72(1), 117 – 122.
[12] F Ramirez; S Dershowitz. *J. Org. Chem*, **1957,** 22(1), 41 – 45.
[13] R A Aitken; J I Atherton. *J. Chem. Soc., Perkin Trans. 1*, **1994,** 10, 1281 – 1284.
[14] K Gerald; N Gunter; S Katica; B Matthias; T Ruth; R Petra. European Patent EP 0421561, 1994.
[15] National Committee for Clinical Laboratory Standards. Method for Antifungal Disk Diffusion Susceptibility Testing of yeasts: Approved Guidelines M44-A Wayne, PA, USA : NCCLS, (2004).
[16] R M Cowper; L H Davidson. *Org. synth.* **1943,** 2, 480.
[17] A Walkowiak; R Wakiec; G Bontemps; R Andruszkiewicz. *J. Enz. Inhib. Med. Chem*, **2005,** 20(5), 439 - 447.
[18] O Grummitt; E I Becker; C Miesse. *Org. Synth*, **1949,** 29, 11.