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In Vitro Antioxidant Evaluation of Novel Anhydride From Stobbe Dicondensation : A Green Chemistry Approach

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Abstract
The use of antioxidants in treatment of oxidative stress-related pathologies is a possible therapeutical strategy for the future. Synthesised product with antioxidant properties could trigger this objective. In present communication, one pot synthesis of acid esters (1) by Stobbedicondensation of alkylidene/arylidene succinates with aldehydes or ketones and their subsequent hydrolysis to diacids (2), namely Fulgenic acids were reported. The Stobbedicondensation of various aromatic aldehydes or ketones with dimethyl succinate gives different types of cyclized products (3), namely (E)-3-(diphenylmethylene)-4-(1-phenylethylidene)dihydrofuran-2,5-dione(3a), and (3Z,4E)-3-benzylidene-4-(1-phenylethylidene)dihydrofuran-2,5-dione (3b) through green approach. The improved yields of Fulgenic acids and its anhydrides (Fulgides) were observed by the green approach as compared with other classical methods employed so far.Diphenylpicrylhydrazyl (DPPH) was used to assay antioxidant activity of presently synthesized compounds.

Key Words: Green synthesis, Hexamethylenetetramine, Stobbe condensation, Aryl aldehydes and ketones, Antioxidant activity

1. Introduction:

The earlier classical methods for the formation of Fulgenic acid and their anhydride forms [1,2] are more time consuming and involves use of hazardous solvents like benzene, ether etc., and hence are not eco-friendly. Present work describes an eco-friendly one pot synthesis method for Stobbe condensation with minimal use of solvents. In contrast to extensive use of solvents and hazardous chemicals used in previous methods[3]; currently studied green method requires fewer amounts of dry solid reagents, for the formation of acid esters [4]. Moreover, solvent free condition improves the yield and heat energy consumption by the reaction is also averted.

Stobbe condensation under solvent free condition using hexamethylenetetramine was carried out with dimethyl succinate, aliphatic aldehyde forms acid-esters, ketone and aromatic, which on saponification[5] yielded corresponding diacids (**2**). Organic photochromic compounds such as Fulgenicacids are potential candidates for application in erasable optical information media[6]. This green approach not only increases the product yield, but also maintains and raises its photochromic strength. The Fulgides i.e. anhydrides (cyclized forms) are the promising materials in optical memory devices, optical switches and sensors, especially dyes and inks. These are representative class of photochromic organic[7, 8, 9]molecules which exhibits several interesting properties for diverse applications in fields such as data storage or high resolution spectroscopy. The anhydride products are prepared by cyclisation of dicondensationdiacids by using silica and perchloric acid.

Fulgenic acid is bioactive monomer against cancer so it is acting as anticancerous compound[10].The antioxidant is a molecule that inhibits oxidation of biomolecule. Oxidation is a chemical reaction that can produce free radicals leading to chain reactions that may damage cells and initiates diseased condition [11]. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, water soluble antioxidants react with oxidants in the cells cytosol and the blood plasma, while lipid soluble antioxidants protect cell membrane from lipid peroxidation[12]. In presently study, we determined the solubility property of Fulgenic acid esters (**1**).

2. Materials and Methods:

2.1 Reagents

The following reagents were used for experiments were obtained from Merck. Dimethyl succinate, hexamethylenetetramine, benzophenone, benzaldehyde, anhydrous methanol, ethylene dichloride, sulphuric acid, alcoholic potassium hydroxide, petroleum ether, n-hexane, etc. All the above solvents were purified by the reported procedures[13]. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, USA. All other chemicals used were of high analytical grade.

2.2 Instrumentation:-

The infrared spectra were obtained on a Bruker AVANCE 520 Fourier transform Infrared spectrometer using KBr pellets from SAIF Punjab University Chandigarh, India. High resolution ^{1}H-NMR spectra was recorded on a BrukerAvance II 400 MHz spectrometer in D_2O with TMS as an internal standard. Melting points were measured on a digital Electrothermal 9100 Melting Point Apparatus and reported without correction. Ultra violet and visible spectra were measured for 10^{-4} M in toluene solution. The pH-metric titrations were conducted in aqueous ethanol (50:50, v/v) on an automatic recording Electronic Corporation of India limited (ECIL) pH-meter (Model pH 821)

having a glass-calomel electrode assembly. Molecular weights of acidic products were determined by titrimetric method as their equivalent weights. The general procedure for Stobbe condensation and saponification of Stobbe condensation products were similar to those described earlier,and were modified by using green method [14, 15].

2.3 (Material synthesis)

A mixture of dimethyl succinate (9.0 g, 0.09 mole) and aldehydes or ketones were added dropwise to a suspension of Hexamethylenetetramine (12.6 g,0.09 mole).The reaction mixture was ground in mortar and pestle for 10 minutes and allowed to stand for another 20 minutes. Then 3N HCl was added in small amounts. Alcohol was distilled off under reduced pressure and reaction mixture was extracted with ether at room temperature. Acidic substances were separated by using 10% Na_2CO_3. On further acidification, finally it gives acid ester which was again recrystallized with n-Hexane /Benzene petroleum ether. Further on esterification, with anhydrous CH_3OH, ethylene dichloride and conc. H_2SO_4at room temperature it gives diester.

Once again the dieters were mixed with aldehydes or ketones and Hexamethylenetetramine, the same procedure was repeated and recrystallization was done with n-Hexane /Benzene,petroleum ether which gives 2nd acid ester. Finally the obtained 2nd acid ester was saponified with alcoholic KOH at room temperature for 2 hours and followed by acidification and recrystallization which would give a solid crystalline natured diacids(Z)-2-(diphenylmethylene)-3-(1-phenylethylidene)succinic acid(**2a**),(2Z,3Z)-2-benzylidene-3-(1-phenylethylidene)succinic acid (**2b**).

Further these diacids undergo cyclisation in presence of silica and perchloric acid (1:1) to give anhydrides (E)-3-(diphenylmethylene)-4-(1-phenylethylidene)dihydrofuran-2,5-dione **(3a)**, and (3Z,4E)-3-benzylidene-4-(1-phenylethylidene)dihydrofuran-2,5-dione **(3b).**

2.4Spectroscopic Analysis of Anhydrides

2.4. 1 (E)-3-(diphenylmethylene)-4-(1-phenylethylidene) dihydrofuran-2,5-dione (3a)

R3, R4 = Ph, Ph(16.40g, 0.09 mole) ,**R1, R2 = Ph, Me** (10.81 g, 0.09mol).

IR (KBr):3265, 3150, 3410, 1868, 1655, 1590,1442cm^{-1}

^{1}H NMR, δ;7.85(1H, s,J=7.5Hz),7.84(1H,s, J=7.8Hz), 7.24(1H,br, J=7.3Hz), 7.14(1H, J=7.1Hz),
7.39(1H,br, J=7.5Hz), 7.25(1H, s,J=7.5Hz), 7.78(1H, s,J=8.5Hz) ; melting point 324^{0}C (**table 1**).

2.4. 2(Z)-3-(diphenylmethylene)-4-(1-phenylethylidene) dihydrofuran-2, 5-dione (3b)

R1, R2 = Ph, H (9.55g, 0.09 mole),**R1, R2 = Ph, Me**(10.81g, 0.09mol)

IR (KBr): 3458, 2655, 2802, 2364, 1764, 1764-1656, 1558, 1154 cm^{-1}

^{1}H NMR,δ;7.46(1H, s,J=6.5Hz),7.45(1H, s,J=7.5Hz)7.44(1H,s, J=7.8Hz), 7.44(1H,s, J=7.3Hz), 7.38(1H,s, J=7.0Hz), 7.65(1H,s, J=7.7Hz), 7.32(1H,s, J=7.5Hz), 7.46(1H,s, J=7.8Hz), 7.36(1H,s, J=7.65Hz), 6.22(1H,s, J=7.8Hz) , 6.73(1H,s, J=7.8Hz) , δ ; 2.40 (CH_3, s, J=6.5Hz) ;melting point286^{0}C(**table 1**).

2.5 Antioxidant Assay

Diphenylpicrylhydrazyl (DPPH) was used to assay antioxidant activity of presently synthesized compounds in a 96-well plate, by modified method of Fukumoto and Mazza (2000) and briefly described here as follows. In each well, 200 µL of DPPH (150 μM prepared in 80% methanol) was mixed with 1µg (25 µL) of synthesized compound. Blank test and, vehicle control were prepared with equal amount of carrier solvent. Different concentrations (0.2µg, 0.4 µg, 0.6 µg, 0.8 µg, 1.0 µg, 1.25 µg and 2.5 µg) of ascorbic acid (vitamin C) were used to prepare standard plot. All samples were assayed in triplicates and the plate was incubated for 6 h at room temperature. After incubation, the absorbances were measured at 517 nm in a multimode reader (Infinite M200). Percent antioxidant activities were calculated by using following formula,

[(Vehicle control – Test)/Vehicle control] X 100

3. Results and Discussions:

Stobbe condensation generally involves the use of metal alkoxideas a catalyst in refluxing alcohol, particularly, butanol[16]. On the other hand, use of butanol is discarded and instead of that, in this research paper Hexamethylenetetramine, was taken for the reaction. The advantages are short reaction time, good yield, less by-products.

TheFulgides **(3a, 3b)** synthesized using current method is of high purityas compared with classical synthesis. The synthesized Fulgides have specific melting and boiling point, NMR peak values. In previous synthetic methodstremendous heat wasused, this leads impure diacids with less percentage yield [17].

Theyellowish colored crystalline solid natureddiacidprepared by using Benzophenone and Acetophenonewhich on cyclisation givesanhydride (**3a**)exhibited a molecular formula$C_{25}H_{18}O_3$showed characteristic stretching frequencies of aromatic C-H(3265, 3150 cm^{-1}), anhydride C=O (1590, 1442 cm^{-1}) , aromatic -CH (1655 cm^{-1}) ,C=C (1868 cm^{-1}).

Similarly,^{1}H NMR spectrum also showed δ7.85 ,7.84, 7.24,7.14,7.39,7.25,7.78 (**Figure1**).

Thebluish colored crystalline solid natureddiacid preparedby using Acetophenoneand Benzaldehydewhich on cyclisation givesanhydride (**3b**) having molecular formula $C_{19}H_{14}O_3$also showed characteristic stretching frequencies of aromatic C-H(3458, 2655cm^{-1}), anhydride C=O (1764-1656 cm^{-1}) , aromatic -CH (2802, 2364 cm^{-1}) , aromatic C=C (1764 cm^{-1}).Similarly,^{1}H NMR spectrum shows aromatic hydrogen'son the corresponding peak valuesδ;7.46,7.45,7.44,7.44,7.38,7.65,7.32,7.46,

7.36,6.22,6.73,2.40 ;(**Figure 1**).The obtained peak values were sharp & accurate for their corresponding groups which proved the dominancy of green approach on classical method.

Antioxidant potential of a chemical entity having biological importance, as origin of most chronic disease is oxidative stress [18]. Cells under the influence of oxidative stress, inevitably produces reactive radicals, which surpasses over antioxidant machinery and construct damages to biomolecules. In present study, antioxidant potentials of synthesized Fulgides were estimated by using a violet colored free radical DPPH, which upon reduction becomes colorless or pale yellow. DPPH color intensity was inversely proportional to antioxidant potentials of chemical compounds. In **Figure 2**, Fulgides have shown DPPH reducing potential along with positive control ascorbic acid. Highest antioxidant activity (16.03 µg) were recorded by(E)-3-(diphenylmethylene)-4-(1-phenylethylidene)dihydrofuran-2,5-dione**(3a)**may be due to presence of three extra phenyl groups along with methyl. 15.68 µg of antioxidant activity pertaining by (3Z,4E)-3-benzylidene-4-(1-phenylethylidene)dihydrofuran-2,5-dione **(3b)** conferred due to the two extra phenyl groups along with Hydrogen in its structure. Exploration of diverse chemical compound for antioxidative activity is mandatory, as to work on variety of pathogenic circumstances, for example in neurodegenerative diseases an antioxidant must have to cross blood-brain barrier but most of the antioxidant cannot, as like carotenoids[19].The therapeutic efficiency of presently synthesized Fulgides in different in vitromodels is our future strategy to evaluate their potency .

4. Conclusion:

The greener chemical reaction strategy managed to synthesize Fulgenic acid (**2a, 2b)** successfully by simple and efficient means with improved yield.The solvent free Stobbe condensation of aromatic aldehydes and aliphatic, aromatic ketones with dimethyl succinate at room temperature occurred smoothly to give substituted acid esters which on further saponification givediacids. These diacids undergo cyclisation in presence of silica and perchloric acid (1:1) to give anhydrides. This methodologybrought down not only the reaction time but also the uses of hazardous organic solvents (as possible)[20, 21]. The prepared anhydrides (**3a, 3b)** can also be used in the preparation of photosensitive glasses, photosensitive toys and other instruments, Optical data recording like Compact Disc;preparation of photosensitive inks for security purpose andvariable density filters.Further, antioxidative nature of these anhydrides may lead to identification of novel compounds to study in the area of pharmaceuticals.

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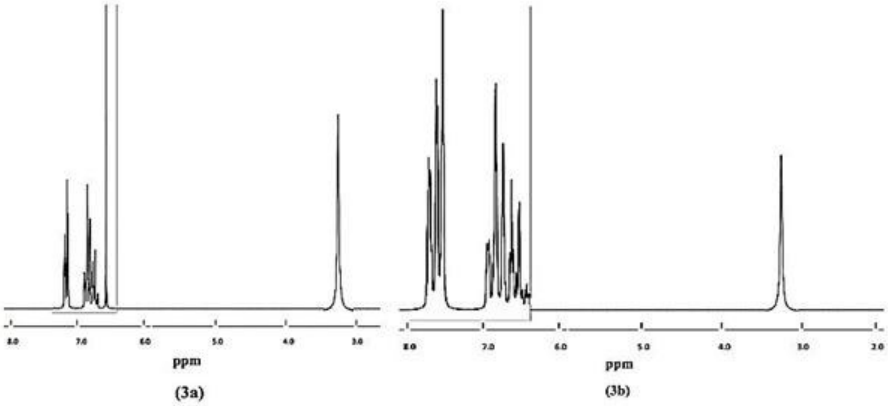

Figure 1: NMR graphical representation of anhydrides

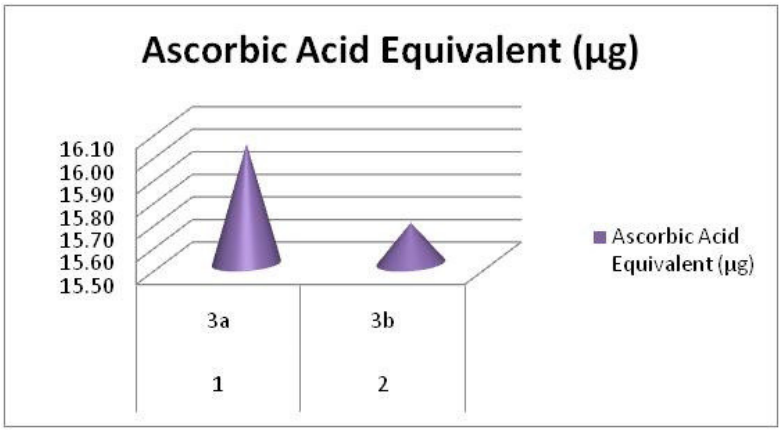

Figure 2: Antioxidentacvtivity of cyclized products (Fulgides)

Table 1: Synthesis of various Anhydrides (**3a–3b**) in the presence of $HClO_4$-SiO_2 by reaction of various aldehydes and ketones at room temperature

Entry	R			Products	Yields (%)		Melting Point (°C)	Molecular Weight	Time[min]
	R_1	R_2	R_3 R_4		Classical	Green[a]			
1	Ph,	Ph	Ph, Me	(**3a**)	62.65	88.29	324	352.38	120
2	Ph,	H	Ph, Me	(**3b**)	69.15	96.14	286	366.41	120

Green[a] refer to the pure isolated products during green synthesis.

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