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Organic Chemistry

Efficiency of High-Acid Fruit Juices as Biocatalysts in the Synthesis of Bis(indolyl)methanes

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Abstract. In modern times, significant effort is directed towards developing environmentally friendly synthetic methods. In this work, we present a green chemistry-based method of the synthesis of bis(indolyl)methanes (BIMs). For the catalyst, we selected the juices of local fruits, specifically Prunus cerasifera, grapes and lemon. To optimize the reaction conditions, the synthesis was conducted in different solvents and at different temperatures. The best results for the reaction were achieved using the juice of unripe *Prunus cerasifera* as a catalyst. The selected solvent is ethanol : water (1.5 : 2.5 mL) mixture and the temperature of 80°C. Using the determined optimal method, we carried out the reactions of indoles with differently substituted aromatic aldehydes. Aromatic aldehydes with electron-withdrawing groups show better yields than those with electron-donating groups. © *2025 Bull. Natl. Acad. Sci. Georg.*

Keywords: bis(indolyl)methanes, fruit juice, green chemistry, eco-friendly

Introduction

Among compounds containing the indole group, a significant portion of interest has been towards bis(indolyl)methanes because they are characterized by a multitude of biological activities (Sharma et al., 2010; Morris et al., 1990). Bis(indolyl)methanes contain two indole groups, which are connected to each other via a single carbon atom, bonded to carbon atoms in 3- and 3'- positions of these indole groups. Such an arrangement of indole groups leads to such important pharmacological activities as antibacterial, antihyperglycemic, anti-inflammatory, anti-cancer, antimicrobial, antiviral and antileishmanial (Shiri et al., 2010). The products resulting from the oxidation of bis(indolyl)methanes can also be used in dyes and calorimetric sensors (Xu et al., 2020). The structures of a few biologically active bis(indolyl)methanes are given in Fig. 1.

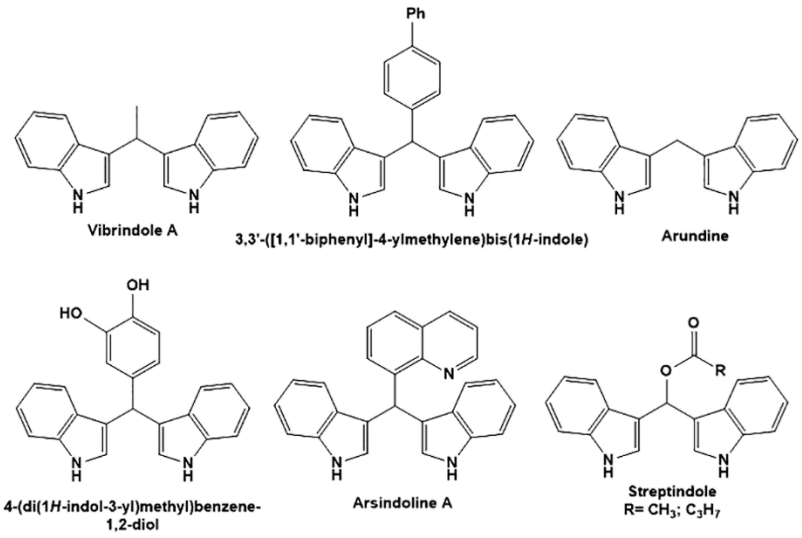


Fig. 1. Biological activity of bis(indolyl)methane's *(*BIMs).

There are many methods to synthesize bis(indolyl)methanes. However, most of these methods have common drawbacks: the necessity of expensive catalysts, the use of volatile and toxic solvents, harsh reaction conditions, etc. Therefore, the synthesis of bis(indolyl)methanes using the concept of green chemistry is interesting to study as it can have important industrial applications.

Our objective was to obtain bis(indolyl)methanes using natural catalysts and eco-friendly/green solvents. The use of fruit juice as catalysts has many advantages such as low cost, ease of acquisition, ease of production, non-toxicity, high reactivity, etc. This route of synthesis helps us avoid the use of toxic catalysts and solvents which are hazardous to human health and the environment.

From the scientific literature, it is known that condensation reactions of indoles with aromatic aldehydes happen under acidic catalysis. Thus, the reaction of condensation was conducted in the juices of different fruits, for which pH ≈ 2–3 (Pal et al., 2013).

Experimental Section

General. All reagents were acquired from Sigma-Aldrich company and used without additional purification. The melting temperatures of target products were measured by a BMP-1 apparatus (BioBase). The ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer, using $CDCl_3$ and DMSO as solvents. The reaction progress was controlled by thin-layer chromatography (TLC) on silica gel 60 F_{254} Merk plates by using ultraviolet illumination and Ehrlich's reagent.

Preparation of Fruit Juice. In the experiments, we used the local breeds of fruit (Georgia): unripe *Prunus cerasifera* ('Guldedava'), unripe grapes (Rkatsiteli and Saperavi) and „Georgian Lemon". The fruits were collected in June. The juice was extracted by squeezing manually, then filtered through filter paper to remove solid residues and a clear portion of the juice was used as a catalyst. The fruit juice that was reserved for experiments was kept at 4°C.

General Procedure for the Synthesis of bis(3-indolyl)methanes. A mixture of 2 mmol of 2-phenylindole and 1 mmol aldehyde was transferred to the round-bottom flask, on which is added 1 mL fruit juice and the mixture of water - ethanol with the ratio of 2.5 mL : 1.5 mL (in total 5 mL mixture, with pH 2.86). The reactant mixture was stirred at 80 ^{O}C for two hours. The progress of the reaction was controlled using TLC.

After completion, the solid crude product was filtered, washed with water and dried by the vacuum pump. Afterward, the target products were crystallized from the ethanol solution.

3,3'-(phenylmethylene)bis(2-phenyl-1H-indole) (3a): white solid, m.p. 264-265°C. IR (KBr, cm-1): 3419, 1599, 1488, 1447, 1341, 1307, 1235, 1018, 830, 741, 698, 667; ^{1}H NMR (400 MHz, DMSO): δ = 11.04 (s, 2H), 7.37 (d, J = 8.1 Hz, 2 H), 7.34 – 7.21(m, 7H) , 7.20 (dd, J = 4.2, 2.3 Hz, 9H), 7.04 – 6.96 (m, 2H), 6.89 (d, J = 8.1 Hz, 2H), 6.71 – 6.63 (m, 2H), 6.06 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ =145.98, 136.88, 135.95, 133.49, 129.29, 128.83, 128.63, 128.52, 127.57, 126.35, 121.26, 121.22, 118.88, 114.81, 111.70.

3,3'-((4-chlorophenyl)methylene)bis(2-phenyl-1H-indole) (3b): white solid, m.p. 236-238 °C. IR (KBr, cm^{-1}): 3389, 1485, 1455, 1444, 1304, 1096, 1012, 820, 766, 742, 718, 694. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (s, 2H), 7.36 (d, J = 8.2 Hz, 2 H), 7.30 - 7.21 (m, 6H), 7.19 – 7.09 (m, 2H), 7.20 (t, *J* = 3.3 Hz, 10H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.88 (t, *J* = 7.6 Hz, 1H), 6.09 (d, *J* = 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.6, 135.98, 135.65, 132.86, 131.69, 130.62, 128.59, 128.41, 128.26, 128.23, 127.59, 121.84, 12162, 119.70, 115.01, 110.67, 39.52.

3,3'-((4-nitrophenyl)methylene)bis(2-phenyl-1H-indole) (3c): yellow solid, m.p. 246-248 °C. IR (KBr, cm^{-1}): 3378, 1592, 1444, 1341, 1236, 1101, 834, 755, 748, 738, 725, 694. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (s, 2H), 8.14 – 8.06 (m, 2H), 7.50 – 7.43 (m, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.21 (s, 8H), 7.27 – 7.12 (m, 4H), 7.01 (d, *J* = 8.2 Hz, 2H), 6.88 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 2H), 6.20 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.95, 146.40, 136.02,135.99, 132.62, 130.04, 128.51, 128.29, 127.86, 123.53, 122.11, 121.21, 119.95, 113.89, 110.87.

4-(bis(2-phenyl-1H-indol-3-yl)methyl)benzaldehyde (3e): yellowish solid, m.p. 253-254 °C. IR (KBr, cm^{-1}): 3395, 1684, 1594, 1456, 1420, 1304, 1211, 1162, 1012, 765, 746, 700, 692. ^{1}H NMR (400 MHz, DMSO): δ = 11.43 (s, 2H), 9.98 (s, 1H), 7.83 (d, *J* = 7.9 Hz, 2H), 7.41 (s, 1H), 7.39 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 2H), 7.32 – 7.21 (m, 10H), 7.03 (t, *J* = 7.6 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 2H), 6.70 (t, *J* = 7.6 Hz, 2H), 6.04 (s, 1H). ^{13}C NMR (100 MHz, DMSO): δ=148.27; 136.77; 136.77; 136.05; 132.98; 130.38; 128.88; 128.66; 128.16; 127.98; 123.31; 121.85; 121.67; 120.70; 119.38; 113.05; 112.07

3,3'-(phenylmethylene)bis(2-(4-bromophenyl)-1H-indole) (3f): white solid, m.p. 239-240 °C. IR (KBr, cm^{-1}): 3377, 1448, 1306, 1235, 1067, 1007,981, 965, 835, 815, 745, 699. ^{1}H NMR (400 MHz, DMSO): δ = 11.36 (s, 2H), 7.41 (d, *J* = 8.1 Hz, 4H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.28 (q, *J* = 9.0, 8.1 Hz, 3H), 7.19 (d, *J* = 7.2 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 4H), 7.02 (t, *J* = 7.6 Hz, 2H), 6.86 (d, *J* = 8.2 Hz, 2H), 6.69 (t, *J* = 7.6 Hz, 2H), 5.95 (s, 1H). ^{13}C NMR (100 MHz, DMSO): δ =145.08, 136.75, 134.67, 132.33, 131.64, 130.47, 129.15, 128.80, 129.29, 126.72, 121.69, 121.16, 121.23, 199.15, 115.04, 111.89.

3,3'-((4-chlorophenyl)methylene)bis(2-(4-bromophenyl)-1H-indole) (3g): white solid, m.p. 259-261 °C. IR (KBr, cm^{-1}): 3391, 1481, 1451, 1306, 1085, 1067, 1009, 746, 724. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (s, 2H), 7.37 – 7.22 (m, 10H), 7.15 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 2H), 7.01 – 6.93 (m, 6H), 6.88 (ddd, *J* = 8.1, 7.0, 1.0 Hz, 2H), 6.02 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.98, 134.48, 132.08, 131.63, 131.53, 130.54, 129.72, 128.56, 128.19, 128.19, 122.05, 121.44, 119.95, 115.32, 110.80.

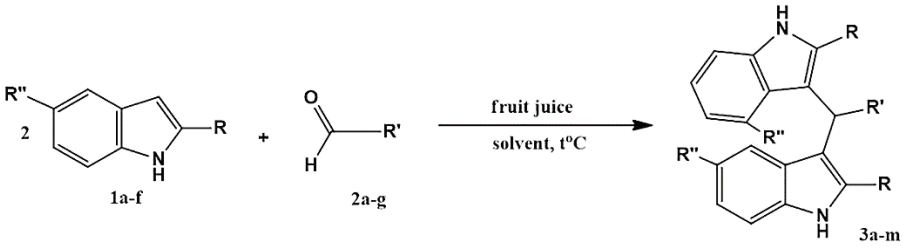
3,3'-((4-nitrophenyl)methylene)bis(2-(4-bromophenyl)-1H-indole) (3h): yellow solid, m.p. 245-247 °C. IR (KBr, cm^{-1}): 3398, 1595, 1451,1481, 1431, 1338, 1305, 1233, 1110, 835, 720, 702. ^{1}H NMR (400 MHz, $CDCl_3$): 8.07 (s, 2H), 8.18 – 8.12 (m, 2H), 7.54 – 7.47 (m, 2H), 7.40 – 7.28 (m, 6H), 7.17 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 2H), 7.01 – 6.94 (m, 4H), 6.97 – 6.90 (m, 2H), 6.89 (ddd, *J* = 8.1, 6.8, 1.0 Hz, 2H), 6.13 (s,

1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.98, 146.60, 135.98, 134.86, 131.63, 131.34, 129.96, 129.74, 127.85, 123.69, 122.47, 122.32, 121.05, 120.19, 114.21, 111.00.

3,3'-(phenylmethylene)bis(2-(4-methoxyphenyl)-1H-indole) (3i): white solid, m.p. 257-259 °C. IR (KBr, cm^{-1}): 3396, 1504, 1491, 1458, 1430, 1248, 1180, 1037, 840, 833, 749, 707. ^{1}H NMR (400 MHz, DMSO): δ = 11.23 (s, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.25 (dd, *J* = 11.7, 7.7 Hz, 7H), 7.13 (d, *J* = 7.4 Hz, 2H), 6.98 (t, *J* = 7.5 Hz, 2H), 6.89 (d, *J* = 8.2 Hz, 2H), 6.80 (d, *J* = 8.3 Hz, 4H), 6.66 (t, *J* = 7.6 Hz, 2H), 5.88 (s, 1H), 3.72 (s, 6H). ^{13}C NMR (100 MHz, DMSO): δ =143.48; 135.99; 135.66; 132.87; 130.64; 128.60; 128.42; 128.37; 128.24; 127.60; 121.85; 121.63; 119.71; 115.02; 110.68

Results and Discussion

The purpose of this work is to research the reactions of different indoles with aromatic aldehydes using eco-friendly solvents and catalysts (Popiashvili et al., 2025). We carried out the synthesis in the juice of different fruits, particularly *Prunus cerasifera*, grape and lemon juice. The general scheme of the reaction is given in Fig. 2.



R_{a-f}= C_6H_5; p-Br-C_6H_4; p-OCH_3-C_6H_4; p-CH_3-C_6H_4; p-NO_2-C_6H_4; p-NO_2-$(C_6H_4)_2$;

R'a-g= C_6H_5-; p-Cl-C_6H_4-; p-NO_2-C_6H_4-; m-NO_2-C_6H_4-; p-CHO-C_6H_4-; p-COOH-C_6H_4-; 3-furaldehyde

R"a,b = H, Cl

Fig. 2. The general way 3,3'-bis(indolyl)methanes (BIMs) synthesis.

In order to determine the optimal conditions for the reaction, we studied the reactions between unsubstituted 2-phenylindoles and benzaldehyde. The experimental conditions are given in Table 1. The synthesis of the target bis(indolyl)methanes was carried out in fruit juices, also in such eco-friendly solvents as water and ethanol, at different temperatures. The reactants, 2-phenylindole and benzaldehyde were taken in a 2:1 molar ratio, respectively.

We tried to carry out the reaction by adding the mixture of fruit juice: water (2:3 volume ratio) to the reactants at room temperature. The reaction progress was controlled by TLC. Even after 72 hours and in the case of all attempted catalysts, the spot of bis-indolyl was observed as only a trace. Thus, the reactive mixture was heated to 60°C for 8 hours. However, the yield of the resulting bis-indolyls was only 4.05-12.11%.

The best result was obtained by heating the mixture of juice of *Prunus cerasifera*: ethanol (95%): water (1:1.5:2.5 volume ratio) to 80°C. In this case, after three hours of starting the reaction, the spot of the initial indole was observeable only as a trace. After treatment and purification of the reaction, the yield of the reaction turned out to be 50-96%. During the optimization of the reaction conditions, we were focused on increasing the yield, while shortening the reaction time. Thus, as optimal conditions, we chose to conduct reactions at 80°C and used the juice of *Prunus cerasifera* as a catalyst (Table 1).

Table 1. Optimization of experimental conditions for synthesis of 3a

Entry	Catalyst* 1 ml	Solvent	Temperature (°C)	Time (h)	Yield (%)
1	*PCJ*	H_2O **	RT 60 80	72 8 8	Trace 12.11 14.22
2	SJ	H_2O**	RT 60 80	72 8 8	Trace 4.15 5.35
3	RJ	H_2O**	RT 60 80	72 8 8	Trace 4.05 5.11
4	LJ	H_2O**	RT 60 80	72 8 8	Trace 8.20 9.31
5	*PCJ*	EtOH – H_2O (1.5 : 2.5, v : v)	RT 60 80	72 3 2	27.15 95.12 96.35
6	SJ	EtOH – H_2O (1.5 : 2.5, v : v)	RT 60 80	72 3 2	13.50 47.17 50.34
7	RJ	EtOH – H_2O (1.5 : 2.5, v : v)	RT 60 80	72 3 2	13.50 46.98 50.10
8	LJ	EtOH – H_2O (1.5 : 2.5, v : v)	RT 60 80	72 3 2	20.17 50.15 80.22

* LJ - Lemon juice; *PCJ -Prunus cerasifera* juice, RJ -unripe Rkatsiteli juice, SJ - unripe Saperavi juice.
**Distilled water is used in this and all other cases; reaction condition: 1a (2mmol), 2a (1mmol), solvent 3ml.

Under the determined optimal conditions, we then carried out the interaction of other indoles with benzaldehydes, particularly, 5-bromo-2-phenylindole, 5-nitro-2-phenyl-1H-indole and 5-nitro-2-diphenyl-1H-indole. The target products were found only in the case of conducting the reaction with 5- bromo-2-phenylindole. In our opinion, the reason for this is the presence of an electron-withdrawing group in the benzene part of the indole group. Due to the nitro group in the phenyl ring, the electron density is significantly diminished at atom C-3 of the indole, which diminishes the nucleophilicity of the indole and negatively affects the reaction (Table 2).

Table 2. Synthesis of bis(indolyl)methanes

Indole	Aldehyde	Catalyst (1 mL)	Solvent	Temperature (°C)	Time (h)	Yield (%)
1b	2a	*PCJ*	EtOH – H_2O (1.5 : 2.5, v : v)	80	2	50.13
1c	2a	*PCJ*	EtOH – H_2O(1.5 : 2.5, v : v)	80	2	95.13
1e	2a	*PCJ*	EtOH – H_2O (1.5 : 2.5, v : v)	80	2	0
1f	2a	*PCJ*	EtOH – H_2O(1.5 : 2.5, v : v)	80	2	0

The substrate scope was investigated with broad range of aldehyde functionality using the optimized reaction conditions. In this, the different electron-donating as well as electron-withdrawing substituents afforded the favored products straight for wardly.

A plausible reaction mechanism for the formation of BIMs (3a-m) is shown in Fig. 3.

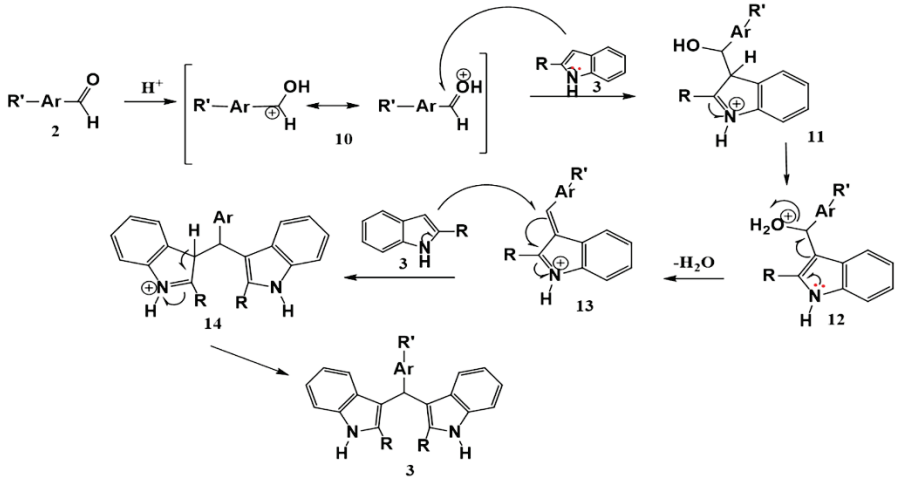


Fig. 3. Probable mechanism for the formation of BIMs.

According to the diagram, as a result of the protonation, the electrophilicity of the carbon atom inside the carbonyl group of the aromatic aldehyde increases. The latter attacks the nucleophilic center of the indole (C-3 carbon atom), and the corresponding monomer product – indolium – is obtained. In the next step, dehydration of the indolium and interaction with a second indole molecule results in the formation of the target product and the release of the catalyst.

Conclusions

In conclusion, we determined the simple and eco-friendly method to synthesize bis(indolyl)methanes using the juice of unripe *Prunus cerasifera.* The developed method is green since we are using natural catalysts and eco-friendly solvents.

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ორგანული ქიმია

მაღალმჟავიანობის მქონე ხილის წვენი, როგორც ბიოკატალიზატორის ეფექტურობა ბის-ინდოლილმეთანების სინთეზში

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თანამედროვე ეპოქაში დიდი ყურადღება ექცევა ნივთიერებების სინთეზის ეკოლოგიურად უსაფრთხო მეთოდების შემუშავებას. წინამდებარე სამუშაოში მოცემულია ბის-ინდოლილ-მეთანების სინთეზის მეთოდი მწვანე ქიმიის კონცეფციაზე დაყრდნობით. მჟავა კატალიზატორად შეირჩა მკვახე ტყემლის, მკვახე ყურძნის და ლიმონის წვენი. რეაქციის პირობების ოპტიმიზაციის მიზნით, სინთეზი ჩატარდა სხვადასხვა გამხსნელსა და სხვადასხვა ტემპერატურულ რეჟიმში. საუკეთესო შედეგი მიღებულ იქნა მკვახე ტყემლის წვენის, როგორც კატალიზატორის, გამოყენებისას. გამხსნელად შეირჩა ეთანოლი:წყალი (1.5 : 2.5 მლ) ნარევი, ტემპერატურა – 80°C-ზე. ჩვენ მიერ დადგენილი ოპტიმალური მეთოდით მოვახდინეთ ინდოლების სხვადასხვა ჩანაცვლებულ არომატულ ალდეჰიდებთან ურთიერთქმედება. არომატული ალდეჰიდები აქცეპტორული ჩამნაცვლებლებით ინდოლებთან ურთიერთქმედებისას იძლევა უკეთეს გამოსავლიანობას ვიდრე ელექტროდონორულით. დადგინდა რეაქციის სავარაუდო მექანიზმი.

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