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A Challenging Classic Coupling: Esterification of Camphoric Acid - a Steric-Hindered Polar Carboxylic Acid and Solanesol - a Long-Chain Nonpolar Alcohol

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Abstract: We have developed two esterification strategies for a challenging coupling between camphoric acid and solanesol to achieve a hybrid natural product - solanesyl camphorate. Both synthetic strategies applied the classic activation mode of carboxylic acid group by anhydride formation to overcome the difficulties caused by steric hindrance and the difference in polarity of two reactants. The first method took advantages of easy prepared camphoric anhydride from camphoric acid, whereas the second one allowed direct esterification via *in situ* anhydride formation. Moreover, no solvent is required in synthetic process. This work would provide greener approaches for syntheses of hybrid esters from similar natural products.

Keywords: Solanesyl Camphorate, antiseptic, wound healing , challenging coupling, solvent-free.

1. Introduction

Along with multiple organ failure, infection accounts for 80% of late deaths in hospital after trauma [1]. Therefore, it is ideal to have a way to simultaneously kill or prevent the development of infectious agents and speed up wound healing process in trauma treatment [2]. Camphoric acid, a dicarboxylic acid derived from cleavage oxidation of a natural terpenoid camphor [3], has been shown to have considerable antiseptic power against the germs of putrefaction and pathogenic organisms [4]. On the other hand, solanesol, which is a natural alcohol in tobacco [5], is an essential building block in many compounds with wound healing activity [6]. Therefore, we expect that monosolanesyl camphorate ester (Figure 1) [7], the new hybrid product between camphoric acid and solanesol, would possess both antimicrobial and wound healing activities to synergistically enhance body tissue repairs after injuries. Unfortunately, there has been no report on the synthesis of this ester. Hence, in this research, we described the first synthesis of solanesyl camphorate as a potential biologically active candidate.

Design Plan

To achieve esterification of camphoric acid and solanesol, however, we need to overcome the two challenges. The first one is the steric hindrance caused by the presence of a quaternary carbon in β-position of the less hindered carboxylic acid group and the long hydrocarbon chain of solanesol. Second, the difference in polarity of two reactants possibly

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hinders efficient collision of the starting acid and alcohol (Figure 1). As a result, our initial effort applying the classic Fisher esterification on these two subtrates [8] only provided decomposed products of solanesol. To solve these challenging obstacles, we have applied two esterification strategies, both utilized the classic activation of carboxylic acid via anhydride formation. This activation mode would provide two considerable advantages (Figure 2). First, the corresponding anhydride would be more reactive toward the nucleophilic attack of solanesol. Second, the polarity of camphoric anhydride would be less than that of camphoric acid allowing higher possibility of efficient collision with nonpolar solanesol.

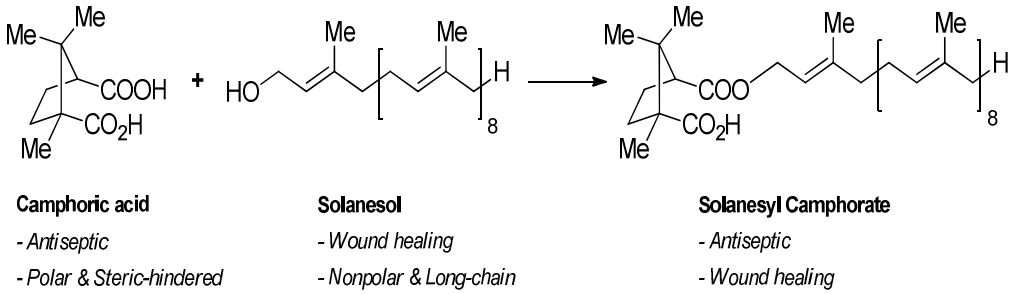


Figure 1. The challenging coupling between Camphoric acid and Solaneso.

1st Strategy: Activation of camphoric acid via pre-formed anhydride

It is trivial to prepare camphoric anhydride by treatment camphoric acid with a dehydrating reagent [9]. As a result, many uncatalyzed couplings of camphoric acid and heat-stable alcohols have been achieved via anhydride activation mode under solvent-free condition by fusion at high temperature [10]. Besides, solanesol was coupled to succinic anhydride [6], a simple cylic anhydride, at room temperature in toxic pyridine [11], and 4-dimethylaminopyridine (DMAP) [12] as the catalyst. Hence, we envisioned that solanesol with its low melting point [13] may serve as both nucleophile and reaction media for esterification with camphoric anhydride in the presence or absence of DMAP. This strategy would avoid using toxic solvents and allow better contact of reactants to compensate for inefficiency of molecular collision caused by steric hindrance.

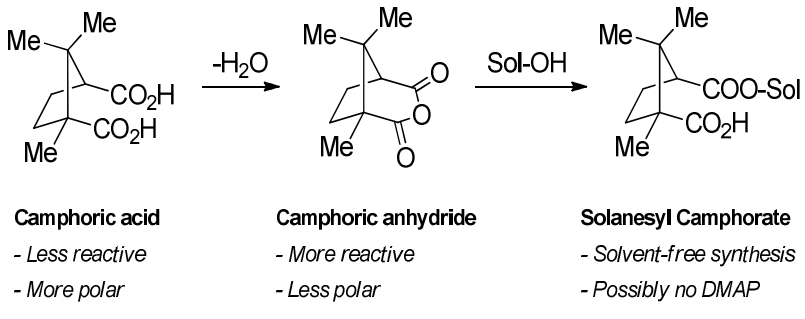
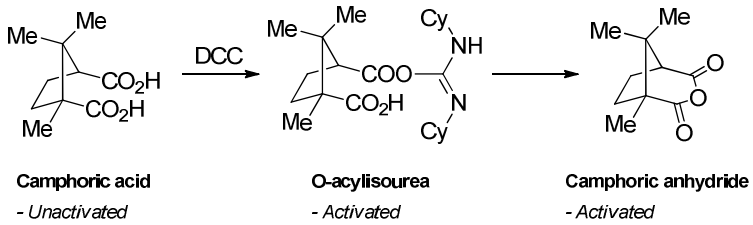


Figure 2. Camphoric acid activation via anhydride formation.

2nd Strategy: Activation of Camphoric acid via *in situ* anhydride

As described in the 1st strategy, activation of camphoric acid via preparation of camphoric anhydride would provide substantial advantages. Therefore, the strategy of activating carboxylic acid groups via *in situ* anhydride formation would be even more advantageous because there is no need for a separate process to synthesize and purify camphoric anhydride. Hence, we considered Steglich esterification [14] - a simple and effective method for challenging combinations of two ester building blocks [15]. In this reaction, a monocarboxylic acid is converted to O-acylisourea [16] by the coupling with N, N'-dicyclohexylcarbodiimide (DCC) and subsequently to its corresponding anhydride [17] both with enhanced activity toward nucleophiles. Because camphoric acid has two closed carboxylic acid groups, the in situ formation of anhydride from the corresponding O-acylisourea is obviously expected (Figure 3). Moreover, this direct esterification strategy may be accomplished under solvent-free condition due to anhydride formation as described in the 1st strategy.



DCC: N,N'-dicyclohexylcarbodiimide; Cy: Cyclohexyl

Figure 3. Camphoric acid activation via *in situ* anhydride formation.

2. Experiment

General information

Camphor, solanesol 95%, DCC, and DMAP was purchased and used without further purification. Column chromatography was accomplished on silica gel. Thin-layer chromatography (TLC) was performed on TLC Silica gel 60 F_{254}. TLC visualization was performed by $KMnO_4$ and/or hydroxylamine/iron (III) chloride. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker BioSpin GmbH spectrometer at a frequency of 500 MHz and 126 MHz, respectively. Data for ^{1}H NMR are reported as follows: chemical shift (δ, ppm), multiplicity (*s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, and *m* = multiplet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift; no special nomenclature is used for equivalent carbons.

2.1. Preparation of camphoric acid and camphoric anhydride

Camphoric acid and camphoric anhydride were synthesized according to ref [3] and [9], respectively. The ^{1}H NMR and ^{13}C NMR spectra were then compared to the published spectra in AIST Spectral Database for Organic Compounds (SDBS number: 6794) for Camphoric acid and ref [10] for Camphoric anhydride.

2.2. Synthesis of Solanesyl Camphorate via pre-formed Camphoric anhydride

a. General procedure for reaction optimization by ^{1}H NMR

A vial containing a mixture of camphoric anhydride (20.0 mg, 0.11 mmol), solanesol 95% (76.2 mg, 0.115 mmol), with or without DMAP (13.4 mg, 0.11 mmol), and 1 mL of solvent except for the cases of solvent-free

esterification was heated at desired temperature. After the completion of reaction (entries with solvent were concentrated under vaccuum), 10.0 mg of internal standard (*p*-methylanisole) was added. The resultant mixture was then subjected to ^{1}H NMR measurement.

b. Synthesis and purification of solanesyl camphorate

A vial containing camphoric anhydride (91.1 mg, 0.50 mmol), solanesol 95% (347.1 mg, 0.52 mmol), and DMAP (61.1 mg, 0.50 mmol) was heated at desired temperature (50^{o}C and 90^{o}C). The progress of reaction was monitored by TLC. After the completion of reaction, the resultant mixtures at 50^{o}C and 90^{o}C were purified by column chromatography eluting with solvent system (hexane:ethyl acetate:dichloromethane = 90:9:1) to afford 327.1 mg and 360.0 mg of product, respectively.

2.3 Synthesis of solanesyl camphorate via in situ Camphoric anhydride formation

a. General procedure for reaction optimization by ^{1}H NMR

Traditional Steglich direct esterification

To a vial containing a mixture of camphoric acid (30.0 mg, 0.15 mmol), solanesol 95% (47.3 mg, 0.07 mmol), DCC (30.9 mg, 0.15 mmol), and DMAP (9.2 mg, 0.075 mmol) was added 1 mL of solvent. The mixture was then stirred at room temperature for 72 h. After that, solvent was removed under vaccum. To the remaining solid, 10.0 mg of internal standard was added. Dissolve the mixture in 1 mL of $CDCl_3$, filter off undissolved solid. The filtrate was then subjected to ^{1}H NMR measurement.

Solvent-free direct esterification

A vial c ntaining a mixture of camphoric acid (30.0 mg, 0.15 mmol), DCC (30.9 mg, 0.15 mmol), Solanesol 95o% (63.0 mg, 0.095 mmol), with or without DMAP (12.2 mg, 0.10 mmol) was heated at desired temperature. After the completion of reaction, 10.0 mg of internal standard was added. Dissolve the resultant mixture in 1 mL of $CDCl_3$, filter off undissolved solid. The filtrate was then subjected to ^{1}H NMR measurement.

b. Synthesis and purification of Solanesyl Camphorate

A mixture of camphoric acid (60.0 mg, 0.30 mmol), DCC (61.8 mg, 0.30 mmol), solanesol 95% (126.0 mg, 0.19 mmol), and DMAP (24.4 mg, 0.20 mmol) was heated at 80^{o}C. The progress of reaction was monitored by TLC. After the completion of reaction, the mixture is dissolved in CH_2Cl_2, filtered off the precipitate, and removed the solvent under vacuum. The resultant mixture was then subjected to purification by column chromatography eluting with solvent system (hexane:ethyl acetate:dichloromethane = 90:9:1) to afford 115.0 mg of purified product.

TLC: R_f = 0.09 in solvent system (hexane:ethyl acetate:dichloromethane = 90:9:1). Visualized by $KMnO_4$.

2.4. Spectroscopic data of solanesyl camphorate

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.36 (*td*, *J* = 7.1, 1.1 Hz, 1H, 1C=C*H*), 5.15 – 5.07 (*m*, 8H, 8C=C*H*), 4.61 (*qt*, *J* = 12.6, 6.3 Hz, 2H, O-C*H*-$_2$), 2.80 (*t*, *J* = 9.4 Hz, 1H, OOC-C*H*), 2.54 (*td*, *J* = 12.5, 7.5 Hz, 1H), 2.21 (*tdd*, *J* = 10.1, 7.9, 2.9 Hz, 1H), 2.10 – 1.95 (*m*, 32H, 8CH_2-CH_2), 1.82 (*dddd*, *J* = 16.3, 12.8, 8.1, 4.3 Hz, 1H), 1.71 (*s*, 3H, 1CH_3), 1.67 (*d*, *J* = 0.9 Hz, 3H, 1CH_3), 1.60 (*s*, 24H, 8CH_3), 1.55 – 1.49 (*m*, 1H), 1.27 (*s*, 3H, 1CH_3), 1.25 (*s*, 3H, 1CH_3), 0.87 (*s*, 3H, 1CH_3).

^{13}C NMR (126 MHz, $CDCl_3$): δ 180.83, 173.86, 142.39, 135.54, 135.07 - 134.86 (6C), 131.25, 124.47 - 124.11 (8C), 123.59, 118.41, 61.26, 56.10, 52.76, 46.74, 39.83 - 39.66 (5C), 39.55, 32.32, 29.71, 26.82 - 26.63 (7C), 26.30, 25.70, 22.73, 22.55, 21.61, 21.22, 17.69, 16.51, 16.08 - 15.98 (7C).

FTIR (in CH_2Cl_2, cm^{-1}): 2964, 2920, 2852, 1731, 1698, 1669, 1447, 1381, 1264, 1166, 1111, 1085, 1056, 983, 907, 838, 734, 703, 600.

LRMS (ESI): m/z calculated for $[M-H]^-$: 811.66. Found 811.81

3. Results and discussion

3.1. 1st Strategy: Activation of Camphoric acid via pre-formed anhydride

Our first examinations of the esterification between camphoric anhydride and solanesol has been presented in Table 1. In the absence of DMAP, no detectable amount of ester was observed in CH_2Cl_2 after 2 days at room temperature (Table 1, entry 1). On the other hand, only 24% yield of solanesyl camphorate has been obtained even when using 1.0 equivalence of DMAP as the activator (Table 1, entry 2). Therefore, the remaining amount of anhydride in entry 2, table 1 as detected by ^{1}H NMR may indicate that either room-temperature condition is not sufficient to provide energy for reactants to overcome activation barrier or dilution by a solvent hinders molecular collision and subsequently results in low reaction efficacy.

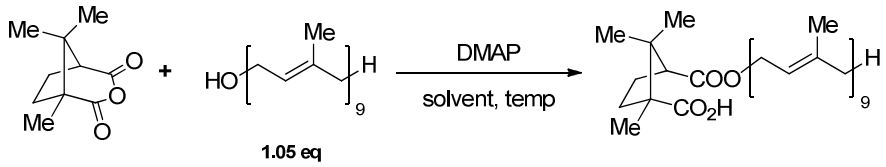


Table 1. Synthesis of Solanesyl Camphorate via pre-formed anhydride.

Entry	DMAP (eq)	Solvent	Temp, °C	Time (h)	NMR yield (%)[a]
1	---	CH_2Cl_2	rt	48	0
2	1.0	CH_2Cl_2	rt	48	24
3	1.0	---	50	24	94 (80[b])
4	1.0	---	70	18	99
5	1.0	---	80	13	98
6	1.0	---	90	8	99 (89[b])
7	---	---	90	24	43

eq: equivalence; DMAP: 4-dimethylaminopyridine; rt: room temperature; eq: equivalence

[a] Determined by ^{1}H NMR analysis with *p*-methylanisole as internal standard; [b] Isolated yield

Based on this analysis, we performed esterification under solvent-free condition with the discussed substantial advantages. As a result, the reaction at 10 °C higher than the melting point of solanesol [13], has provided 94% ^{1}H NMR yield (80% isolated yield) of the desired ester after 48 hours (Table 1, entry 3). A followed study on reaction temperature furnished up to 99% yield after 8 hours (entries 4-6). The reaction in entry 6 was then scaled up to give 89% isolated yield of solanesyl camphorate. We also examined solvent-free esterification without DMAP (Table 1, entry 7, 43% yield). This moderate yield was probably due to the sublimation of camphoric anhydride observed during the reaction process without the presence of DMAP.

3.2. 2nd Strategy: Activation of Camphoric acid via in situ anhydride

Due to ready *in situ* formation of camphoric anhydride from camphoric acid and DCC [18], we anticipated that the condition for direct coupling of camphoric acid and solanesol would be similar to that of camphoric anhyride and solanesol. Our initial examination of traditional Steglich esterification in two common solvents for this method (CH_2Cl_2 and DMF) [14] only afforded low yield of ester after 3 days even though 2.1 equivalences of camphoric acid were used (Table 2, entries 1, 2). With the advantage of the solvent-free esterification achieved, we performed the direct esterification without solvent. By this simple modification, the coupling proceeded smoothly to give up to 100% yield of ester (Table 2, entries 3-5). From this result, direct esterification was performed at 80°C to achieve 75% isolated yield of solanesyl camphorate.

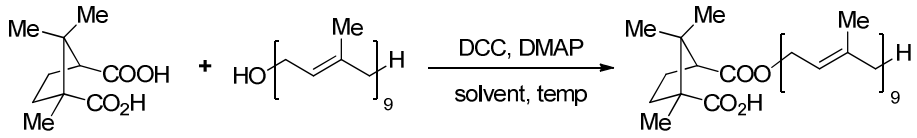


Table 2. Synthesis of Solanesyl Camphorate via *in situ* anhydride formation

Entry	CA (eq)	DCC (eq)	DMAP (eq)	Solvent	Temp, °C	Time (hour)	NMR yield (%)[a]
1	2.1	2.1	1.05	CH_2Cl_2	rt	72	12
2	2.1	2.1	1.05	DMF	rt	72	1
3	1.6	1.6	1.05	---	80	8	100 (75[b])
4	1.6	1.6	1.05	---	90	5	97
5	1.6	1.6	1.05	---	100	2	100
6	1.6	1.6	---	---	90	24	79

eq: equivalane; CA: Camphoric acid; DMF: dimethylformamide; THF: tetrahydrofuran

[a] Determined by ^{1}H NMR analysis with p-methylanisole as internal standard; [b] Isolated yield

Interestingly, in the absence of DMAP, yield of solanesyl camphorate ester was 79% as detected by ^{1}H NMR (Table 2, entry 6). The higher yield in this case compared to entry 7, Table 1 can be explained by the use of an excess amount of camphoric acid compared to an only nearly equimolar mixture of camphoric anhydride and solanesol (Table 1, entry 7). In addition, the own reactivity of *O*-isoacylurea may partially account for this difference. This finding might open the possibility to exclude the use of toxic DMAP [19] for esterification of similar natural products.

4. Conclusion

We have successfully achieved the challenging esterification between camphoric acid and solanesol by two strategies: via pre-formed camphoric anhydride and *in situ* anhydride formation both excluded the use of organic solvents. The *in situ* anhydride formation offers a direct synthetic route to the hybrid compound solanesyl camphorate. Moreover, the good yield of derised ester in the absence of DMAP may open up possibility for greener esterification methodology of similar natural products.

Acknowledgement

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[18] A mixture of Camphoric acid and DCC (1.1 eq) was stirred in THF. A white precipitate appeared instantly (possibly dicyclohexylurea). A thin-layer chromatography analysis of mixture was performed by selective TLC stain of acid anhydride (hydroxylamin/Iron (III) chloride). The mixture gave spot with the same retention factor (R_f) as Camphoric anhydride. In addition, the formation of Camphoric anhydride was also confirmed by ^{1}H NMR measurement of the mixture.

[19] Material Safety Data Sheet, Fisher Scienctific (Cat Number: BP596-110).

Ester hóa acid phân cực, án ngữ không gian camphoric và alcohol mạch dài, không phân cực solanesol

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Tóm tắt: Chúng tôi đã phát triển hai phương pháp este hóa giữa acid camphoric và solanesol để thu được sản phẩm solanesyl camphorate. Cả hai cách tổng hợp này đều áp dụng phương pháp hoạt hoá acid carboxylic thành anhydride để vượt qua sự cản trở không gian và sự khác biệt về độ phân cực của hai chất phản ứng. Phương pháp đầu tiên tận dụng khả năng dễ chuyển hoá thành anhydride của acid camphoric, trong khi phương pháp thứ hai cho phép este hóa trực tiếp thông qua sự hình thành anhydride trong quá trình phản ứng. Hơn nữa, hai phương pháp này không cần sử dụng dung môi. Các quá trình này góp phần vào hệ thống các phương pháp tổng hợp của các este lai từ các sản phẩm tự nhiên tương tự.

Từ khoá: Solanesyl Camphorate, kháng khuẩn, làm lành vết thương, phương pháp ghép mạch, phản ứng không dung môi.