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LETTERS

Syntheses of isocitric acid derivatives and biological evaluation

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Abstract

Isocitric acid methyl esters and their derivatives have been synthesized and their biological activities, superoxide release inhibition and tumor necrosis factor-alpha (TNFα)'s release inhibition, have been studied. Linoleic and linolenic acid derivatives showed strong activity against TNFα's release inhibition comparable to sarcophytol A and cryptoporic acid E.

Keywords: biologically active compounds; carboxylic acids and derivatives; esters; terpenes and terpenoids.

We have previously reported the isolation of cryptoporic acids A–F from the fungus *Cryptoporus volvatus* and established their structures based on high resolution 2D NMR techniques, X-ray analysis, and chemical degradations.[1–5] It is very interesting to note that cryptoporic acids exhibit strong superoxide release inhibition and anti-tumor promotion activities.[5] The structures are unusual; namely, they are drimane-type sesquiterpenes linked to isocitric acid moiety with an ether bond.[6–10] We were interested in their biological activities and planned to prepare synthetic substitutes to the natural products.[11] We first intended to prepare isocitric acid with the same stereochemistry as natural products. Then fatty acids were attached to the isocitric acid and biological activities of these esters were evaluated. We now report our preliminary results.

Since Seebach et al. reported the alkylation of malic acid and synthesis of isocitric acid,[12] we have modified their methodology to prepare monocarboxylic acid derivatives[13] (Scheme 1). The allylated compound **2** of (*R*)-(+)- or racemic malic acid was protected with the MOM group and subjected to ozonolysis. The Jones' oxidation of aldehyde **4** afforded acid **5**, which was converted to trichloroethyl ester **6** using DCC. The MOM group was deprotected by TMSBr and fatty acids were used for the preparation of esters, whose trichloroethyl group was removed under Zn–AcOH–MeOH conditions to afford **8**, **9** and **10**.[14] Trimethyl isocitrate (**11**)[14] was synthesized by methylation (CH_2N_2) of acid **5** and deprotection of the MOM group in 65% yield from **5**.

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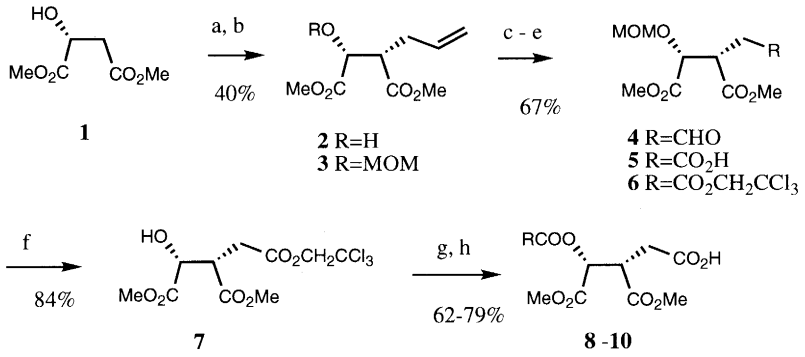
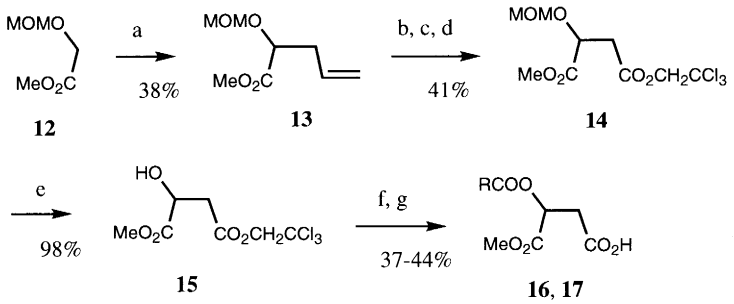


Scheme 1. *Reagents and conditions*: (a) LDA, $BrCH_2CH{=}CH_2$; (b) MOM–Cl, EtN^iPr_2; (c) O_3, CH_2Cl_2–MeOH, then SMe_2; (d) Jones; (e) DCC, $HOCH_2CCl_3$, DMAP; (f) $BrSiMe_3$, CH_2Cl_2; (g) RCOOH, DCC, DMAP, CH_2Cl_2; (h) Zn, AcOH, MeOH

Malic acid derivatives were similarly prepared (Scheme 2). The MOM-protected methyl glycolate was allylated, and similarly converted to methyl trichloroethyl ester of the malic acid **15**. The esterification with fatty acids using DCC and deprotection afforded **16** and **17**.[14]



Scheme 2. *Reagents and conditions*: (a) LDA, $BrCH_2CH{=}CH_2$; (b) O_3, CH_2Cl_2–MeOH, then SMe_2; (c) Jones; (d) DCC, $HOCH_2CCl_3$, DMAP; (e) $BrSiMe_3$, CH_2Cl_2; (f) RCOOH, DCC, DMAP, CH_2Cl_2; (g) Zn, AcOH, MeOH

Because the natural products exhibit strong superoxide release inhibition activities,[4,5] these compounds were subjected to the same tests.[4,5] The results are listed in Table 1.[15] Among those tested, compound **16** was the most effective (IC_{50} 0.39 μM). The malic acid derivatives were 10 times more effective than isocitric acid derivatives. The linoleic acid derivatives were more than twice as strong as linolenic acid derivatives.

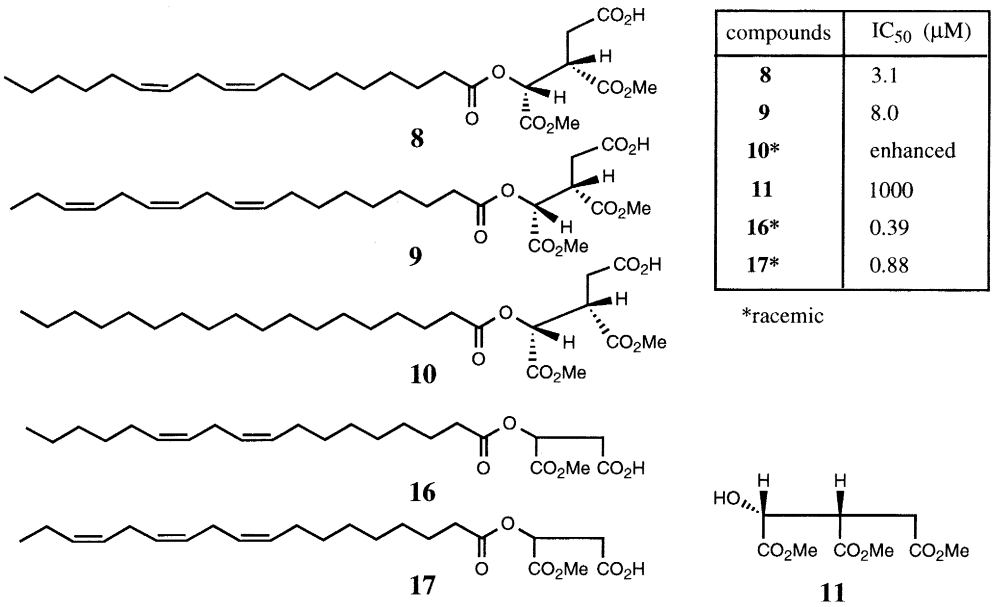
Compounds **8** and **9** were further subjected to inhibition tests of TNFα's release with okadaic acid by anti-tumor promoters on BALB/3T3 cells.[5,16] As shown in Fig. 1, both compounds are as strong as sarcophytol A (**18**) and cryptoporic acid E (**19**). Therefore, it is very interesting to note that the relative positions of the hydrophilic and lipophilic parts are very important, and that the lipophilic part can be substituted by fatty acids to show anti-tumor promoter activity.

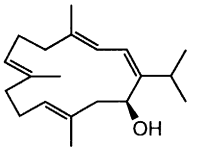
Thus, we have prepared fatty acid derivatives with isocitric and malic acid moiety and evaluated their biological activities, and two of them (**8** and **9**) were as effective as sarcophytol A (**18**) and cryptoporic acid E (**19**) in inhibition of TNFα's release.

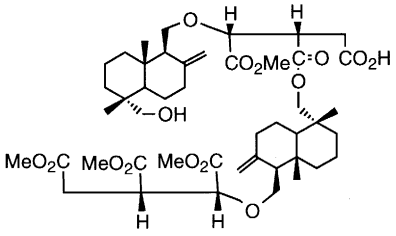
Table 1
Superoxide release inhibition activity against guinea pig macrophage

compounds	IC_{50} (μM)
8	3.1
9	8.0
10*	enhanced
11	1000
16*	0.39
17*	0.88

*racemic




Sarcophytol A (**18**)


Cryptoporic acid E (**19**)

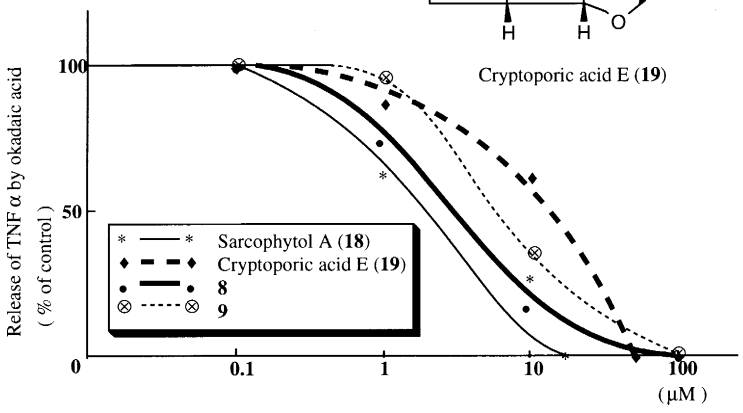


Fig. 1. Inhibition of TNFα's release with okadaic acid by anti-tumor promoters on BALB/3T3 cells

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