☘Preparation and Characterization of Derivatives of Isoricinoleic Acid and Their Antimicrobial Activity

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ABSTRACT

Methylisoricinoleate (methyl-9-hydroxy-*cis*-12-octadecenoate) (I) has been derivatized to yield new fatty acid derivatives analogous to those obtained from the ricinoleic acid of castor oil. These derivatives include 1,9-dihydroxy-12-octadecene (II); 1,9-diacetoxy-12-octadecene (III); 12,13-epoxy-1,9-diacetoxyoctadecane (IV); 9-cyanoethoxy-1-hydroxy-12-octadecene (V); 1-morpholine-9-hydroxy-12-octadecene (VI), and 1-morpholine-9-cyanoethoxy-12-octadecene (VII). Structures of these compounds were established by chemical and spectral data. The compounds V, VI and VII showed antifungal activity against *Alternaria* sp., *Helminthosporium* sp., *Penicillium citrinum, Fusarium oxysporum, Aspergillus ochraceous, A. flavus, A. niger, Actinomyces* sp. and *Cladosporium harbarum.*

INTRODUCTION

The literature has many reports of the use of castor oil's ricinoleic fatty acid (12-hydroxy-9-octadecanoic) and its derivatives in a variety of industrial applications. Isoricinoleic acid (9-hydroxy-12-octadecenoic acid) possesses a γ-hydroxyolefinic group, whereas ricinoleic acid possesses a β-hydroxyolefinic function. It has been reported that better low-temperature plasticizing properties are obtained with compounds having a hydroxyl group at the C9 rather than the C12 position (1,2). Further, isoricinoleic acid, which occurs to the extent of 60-78% in the seed oils of some species of the Apocynaceae family (3-5), has the potential to be used as an alternative source of oleochemicals similar to those derived from castor oil. Seed oils with a major fatty acid of unusual structure could become a source of valuable chemical intermediates for the oil industry. Therefore, with a view to exploring the possible use of isoricinoleic acid, a number of derivatives similar to those reported from castor oil products have been prepared, and the N-derivatives have been subjected to antimicrobial screening.

EXPERIMENTAL PROCEDURES

Infrared (IR) spectra were recorded with a Pye-Unicam SP 3-100 spectrophotometer on KBr disc or as liquid films. Nuclear magnetic resonance (NMR) spectra in $CDCl_3/CCl_4$ were run on a Varian A60 spectrometer using trimethyl silane (TMS) as internal standard. Thin layer chromatography (TLC) was performed on plates coated with 0.25 mm thick silica gel (20 x 5 cm plates) using petroleum ether/ether/acetic acid and petroleum ether/ether/methanol/acetic acid as developing solvents. Spots were visualized by charring after spraying with a 20% aqueous perchloric acid. Petroleum ether refers to the fractions with b.p. 40-60 C. Compounds were purified by column chromatography using silica gel and petroleum ether, ether and methanol as eluting agents.

Isoricinoleic acid was isolated from the saponified oil of *Wrightia tinctoria* (3) by Gunstone's partition procedure (6). Methyl ester I was prepared by using methanolic sulphuric acid (0.25 M).

Reaction of I with Lithium Aluminum Hydride (LAH)

A solution of I (5 g) in dry ether (25 ml) was added drop by drop to a solution of LAH (3 g) in 100 ml dry ether at ice bath temperature (five min), and held for another two min; the content of the reaction flask then was worked up with ether, acidified and filtered. The white solid compound II crystallized from petroleum ether (b.p. 40-60 C) melted at 50-52 C (90% yield). Analysis: Found: C, 75.75; H, 12.54; as $C_{18}H_{36}O_2$ requires: C, 75.99; H, 12.75%. IR (cm^{-1}): 3400-3300, 3200, 1150, 1075; NMR (δ): 5.4 m (HC=CH-), 2.1 m (2 x OH, D_2O exchangeable), 3.6 m (CH_2OH, CH), 1.2 br s (chain methylene), 0.87 t (terminal methyl).

Reaction of II with Acetic Anhydride

To a solution of II (4 g) in pyridine (80 ml) acetic anhydride (25 ml) was added and the reaction mixture was kept for 24 hr at room temperature. The solvent was evaporated under vacuum, and the content of the flask was worked up with ether and dried over sodium sulphate. Evaporation of ether gave a TLC homogeneous liquid product III (90%). Analysis: Found: C, 71.50; H, 10.60; as $C_{22}H_{40}O_4$ requires: C, 71.69; H, 10.93%. IR (cm^{-1}): 1740, 1380, 1240; NMR (δ): 5.35 m (-HC=CH-), 4.8 m (CH-OAc), 3.9 m (CH_2-OAc), 2.1 br s (2 x $OCOCH_3$).

Reaction of III with *m*-Chloroperbenzoic Acid (*m*-CPBA)

Solution of III (2 g) in chloroform (50 ml) was allowed to react with *m*-CPBA (1.2 g) at room temperature for eight hr. Solvent was removed under reduced pressure, agitated with 10% $NaHSO_3$ (20 ml) extracted with ether, and washed with 5% $NaHCO_3$ (20 ml) and water. Sodium sulphate dried liquid product (IV) was obtained in 90% yield. Analysis: Found: C, 68.50; H, 10.24; as $C_{22}H_{40}O_5$ requires: C, 68.71; H, 10.48%. IR (cm^{-1}): 825, 848, 1240, 1360, 1740; NMR (δ): 4.8 m (CH-OAc), 4.0 m (CH_2-OAc), 2.85 m (-CH—CH-), 2.0 br s (2 x $OCOCH_3$).

Reaction of II with Acrylonitrile

To the solution of II (10 g) in dioxane (80 ml), 3% sodium methoxide (10 ml) was added as catalyst. The reaction mixture was heated at 50 C and stirred, and acrylonitrile (35 ml) was added drop by drop; the temperature was kept constant (50 C). After complete addition of acrylonitrile, the temperature was raised to 85 C. After three hr, the reaction mixture was poured into ether. The excess of acrylonitrile precipitated in the form of polyacrylonitrile. The cyanoethylated product dissolved in ether was decanted and washed with 0.1 N HCL and dried over sodium sulphate. Evaporation of ether gave product (V), which was purified by silica gel column using petroleum ether/ether as eluting agents (55%). Analysis: Found: C, 74.67; H, 11.76; N, 4.21; as $C_{21}H_{39}O_2N$ requires: C, 74.72; H, 11.64; N, 4.15%; IR (cm^{-1}): 3300, 2200, 1460, 1360, 1100; NMR (δ): 5.35 m (-HC=CH-), 3.6 m (CH_2OH, D_2O exchangeable), 3.2 m (CH_2-OH), 2.4 m (CH_2-CN).

Reaction of I with Morpholine

Methyl isoricinoleate (I) (20 g) was refluxed with morpholine (11.15 g) for 36 hr following the procedure of Dupuy et al. (1). The content was extracted with ether and washed with water, and a viscous oily product (VI) in 80% yield was obtained. Analysis: Found: C, 71.00; H, 11.04; N, 3.69;

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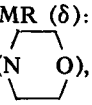
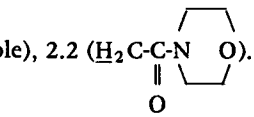
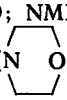
as $C_{22}H_{41}O_3N$ requires: C, 71.49; H, 11.72; N, 3.78%. IR (cm^{-1}): 3400-3300, 1630, 1440, 1260, 1010; NMR (δ): 5.33 m (-HC=CH-), 3.55 m (CH-OH), 3.45 br s (), 2.7 m (OH, D_2 exchangeable), 2.2 ().

Reaction of VI with Acrylonitrile

To a warm solution (50 C) of VI (5 g) in dry dioxane (50 ml) and 3% sodium methoxide (8 ml), acrylonitrile (20 ml) was added drop by drop for 30 min with the temperature constant (50 C). Then the temperature was raised to 85 C and held there for two hr, then cooled and poured into ether. After 3 hr, the excess acrylonitrile was removed in the form of polyacrylonitrile. The ethereal layer was decanted and washed with 0.1 N HCl; evaporation of ether gave product VII as a viscous liquid in 55% yield. Analysis: Found: C, 71.06; H, 11.06; N, 6.46; as $C_{25}H_{44}O_3N_2$ requires: C, 70.87; H, 11.18; N, 6.61%; IR (cm^{-1}): 2200, 1630, 1440, 1260, 1010; NMR (δ): 5.37 m (HC=CH-), 3.60 m (CH-OH), 3.5 br s (), 2.4 m (CH_2-CN).

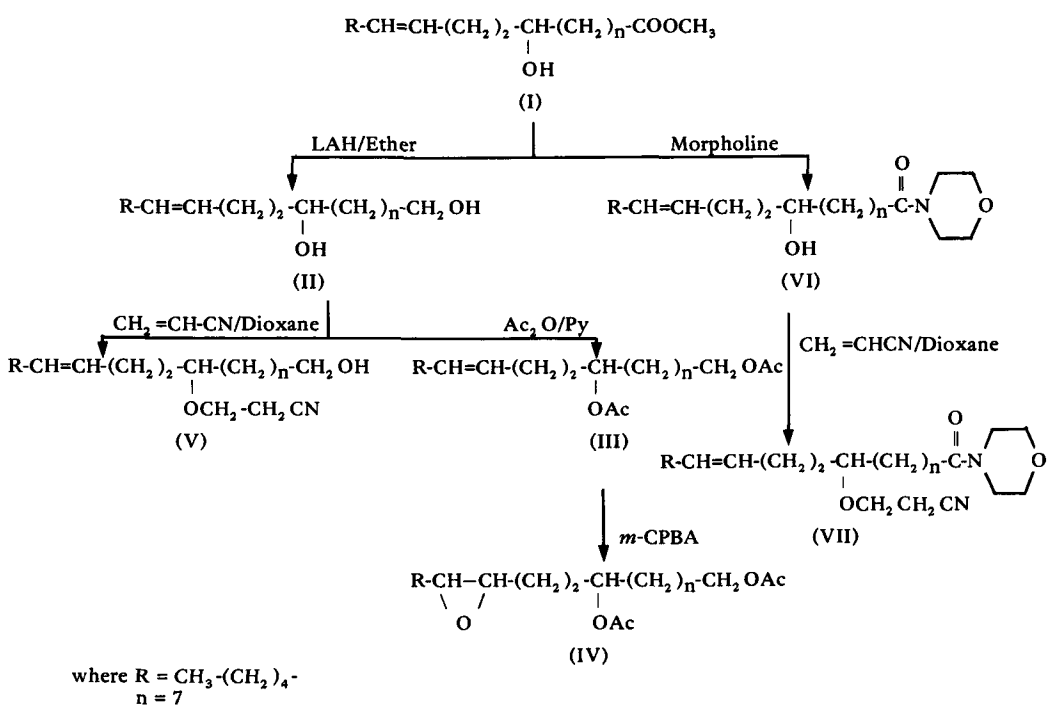
Microbial Activity

One per cent stock solution of V, VI and VII was prepared in acetone. From the stock solution (1%), concentrations of 10 and 100 ppm of V, VI and VII were prepared in distilled water for testing antimicrobial activity in vitro against test fungi, viz., *Alternaria* sp., *Helminthosporium* sp., *Penicillium citrinum*, *Fusarium oxysporum*, *Aspergillus ochraceous*, *A. flavus*, *A. niger*, *Actinomyces* sp. and *Cladosporium harbarum*. These test fungi were isolated from soil by the soil plate method of Warcup (10). Pure cultures of these fungi were maintained in culture tubes on potato dextrose agar. Aseptically, 10 ml of 1% sterilized water agar medium was poured in petri dishes. In one set of petri dishes 2 ml of solutions of each compound were incorporated separately. In another set, 2 ml solution in distilled water of acetone 10 and 100 ppm was added. Then each set was inoculated with test fungi by aseptic transfer from culture tubes to the surface of 1% water agar medium. Petri dishes were incubated at 28-30 C for one week, and radial growth of test fungi was measured. For each treatment 10 replicates were taken. Controls were run simultaneously with and without acetone to observe the complete inhibitory effect of the compounds.

RESULTS AND DISCUSSION

Various chemical transformations involving hydroxy, double bond and ester functions of isoricinoleic acid methyl esters are outlined in Scheme I. These derivatives were characterized by their IR and NMR spectral properties, described in the experimental section.

Methyl isoricinoleate (I) was converted to alcohol (II) in quantitative yield by selective reduction of methylester group with LAH. The isoricinoloyl alcohol (II) on acetylation with acetic anhydride and pyridine at room temperature gave the corresponding diacetate (III) quantitatively, which was converted to epoxydiacetate (IV) by reacting with *m*-CPBA. The addition of acrylonitrile (cyanoethylation) occurs with compounds such as amine, oxine and alcohols, having a labile hydrogen atom. An ester group in hydroxy esters inhibits cyanoethylation (7). However, by addition of sodium methoxide/potassium methoxide in reaction mixture or by converting the interfering methoxy group to morpholine or carbonyl function, the quantitative cyanoethylation was achieved. Cyanoethylation of isoricinoloyl alcohol (II) was very selective, the C9 hydroxy group was converted to cyanoethyl ether (V) in about 50%. A high yield of cyanoethylated product (VII) was obtained when ester I was first converted to morpholine (VI) derivative followed by cyanoethylation.



SCHEME 1

TABLE I

Antimicrobial Activity of Nitrogenous Derivatives

Fungi / Test organisms	Effect of compounds on colonial growth in cm					
		Test compounds/ppm				
		V[a]		VI[a]		VII[a]
	Control[a]	10	100	10	100	10
Alternaria sp.	2.5	1.8	0.0	1.3	0.0	0.0
Helminthosporium sp.	2.0	1.6	0.0	0.0	–	0.0
Penicillium citrinum	1.5	1.0	0.0	1.0	0.0	0.0
Fusarium oxysporium	1.5	0.0	–	0.0	–	0.0
Aspergillus ochraceous	1.0	0.0	–	0.8	0.0	0.0
Aspergillus flavus	1.8	0.0	–	1.6	0.0	0.0
Aspergillus niger	1.7	0.0	–	0.9	0.0	0.0
Actinomyces ap.	0.4	0.0	–	–	0.0	0.0
Cladosporium barbarum	0.6	0.3	0.0	0.2	0.0	0.0

[a]Means of 10 replicates.

Various seed oils, fatty acids and their derivatives are known for their pesticidal (8) and antimicrobial properties (9) and have commercial interest, because they possess broad antimicrobial activity. Of the nitrogenous derivatives of isoricinoleic acid prepared and tested for antifungal activity against nine species of fungi under optimum culture conditions, 10 ppm solutions of V and VI were poor to some classes of fungi whereas VII was very effective. Table I shows the results of the microbial testing of these compounds.

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REFERENCES

1. Dupuy, H.P., R.T. O'Connor and L.A. Goldblatt, JAOCS 35:99 (1958).
2. Dupuy, H.P., R. Calderon, F.R. MeCall, R.T. O'Connor and L.A. Goldblatt, JAOCS 36:659 (1959).
3. Ansari, F.H., G.A. Qazi, S.M. Osman and M.R. Subbaram, Ind. J. Appl. Chem. 34:157 (1971).
4. Siddiqi, S.F., F. Ahmad, M.S. Siddiqi and S.M. Osman, Chem. Ind. (London) 115 (1980).
5. Gunstone, F.D., and L.J. Morris, J. Sci. Food Agric. 10:522 (1959).
6. Gunstone, F.D., J. Chem. Soc. 1611 (1954).
7. Bruson, H.A., Org. Reactions 5:79 (1949).
8. Khan, M.W.Y., F. Ahmad, I. Ahmad and S.M. Osman, JAOCS 60:949 (1983).
9. Novak, A.F., G.C. Clark and H.P. Dupuy, JAOCS 38:321 (1961).
10. Warcup, J.H., Nature 166:117 (1950).

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