Synthesis of C-18 Mixed Acid Diacyl-*sn*-Glycerol Enantiomers[1]

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ABSTRACT

Procedures have been developed for the synthesis of both enantiomeric forms of mixed fatty acid, saturated and polyunsaturated 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols from D-mannitol as starting material. The following diacyl-*sn*-glycerols have been synthesized: 1-Stearoyl-2-linoleoyl-*sn*-glycerol, 1-stearoyl-2-linolenoyl-*sn*-glycerol, 2-linoleoyl-3-stearoyl-*sn*-glycerol and 2-linolenoyl-3-oleoyl-*sn*-glycerol. Their specific rotations, refractive indices, densities, solubilities, carbon and hydrogen analysis and iodine values have been reported.

INTRODUCTION

In order to obtain reference compounds for the natural diacyl-*sn*-glycerols when isolated, it has been necessary to devise methods of synthesis of various types of diacyl-*sn*-glycerols to duplicate the natural products.

The synthesis of optically active 1,2-diacyl-*sn*-glycerols has been attempted by many investigators through the optical resolution of the racemic intermediates possessing acidic or basic groups as precursors of the desired optically active acyl-*sn*-glycerols (1-5). However none of these methods has yielded enantiomerically pure diacyl-*sn*-glycerols. The synthesis of enantiomerically pure 1,2-distearoyl-*sn*-glycerol, 1,2-dipalmitoyl-*sn*-glycerol and 1,2-dimyristoyl-*sn*-glycerol was achieved by Sowden and Fischer in 1941 from 1,2-isopropylidene-*sn*-glycerol (6). This was obtained from naturally occurring D-mannitol by the methods that transfer the asymmetry of the carbon 2 and 5 of D-mannitol to the 1,2-isopropylidene-*sn*-glycerol (7-9). Thus the 1,2-isopropylidene-*sn*-glycerol provides the stereochemical key substance as starting material for the synthesis of optically active 1,2-diacyl-*sn*-glycerols. Howe and Malkin (10) used the procedure of Sowden and Fischer for the synthesis of the corresponding racemic compounds and they were able to improve the method, obtaining higher yields in each stage of synthesis.

All such diacyl-*sn*-glycerols prepared to that date contained solely saturated fatty acid substituents, while the unsaturated acid 1,2-diacyl-*sn*-glycerols had defied attempts at synthesis.

In 1958 Baer and Buchnea (11) reported for the first time the synthesis of 1,2-dioleoyl-*sn*-glycerol and 2,3-dioleoyl-*sn*-glycerol by converting the oleic acid substituents into 9,10-dibromostearic acids and regenerating the double bond with activated zinc after removal of the protective benzyl group from the 3 position by catalytic hydrogenolysis. Two years later Buchnea and Baer (12) reported the synthesis of 1-stearoyl-2-oleoyl-*sn*-glycerol and 1-oleoyl-2-stearoyl-*sn*-glycerol. These methods are readily adapted to the synthesis of monounsaturated acid diacyl-*sn*-glycerols and mixed acid, saturated and monounsaturated diacyl-*sn*-glycerols, but not of course to the synthesis of mixed acid saturated and polyunsaturated, diacyl-*sn*-glycerols.

Recently, Pfeiffer et al. (13) were able to synthesize optically active, polyunsaturated 1,2-diacyl-*sn*-glycerols by employing 2,2,2-trichloroethoxycarbonyl as a protecting group. So

[1]Presented in part at the ISF-AOCS World Congress, Chicago, September 1970.

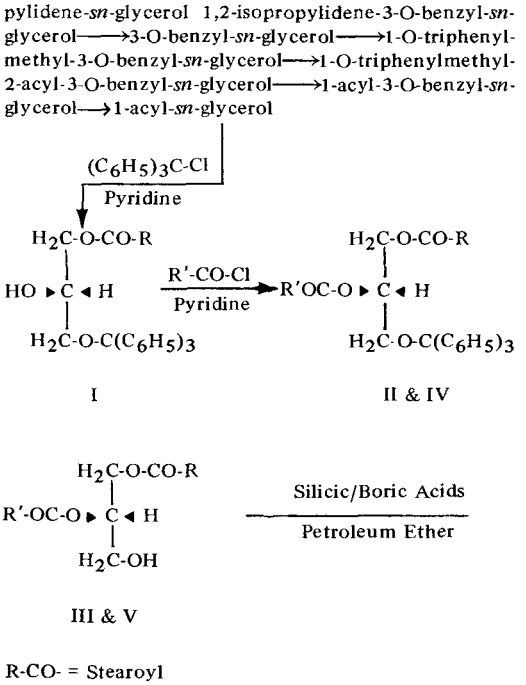


FIG. 1. Synthesis of 1,2-diacyl-*sn*-glycerol from D-mannitol via above intermediates.

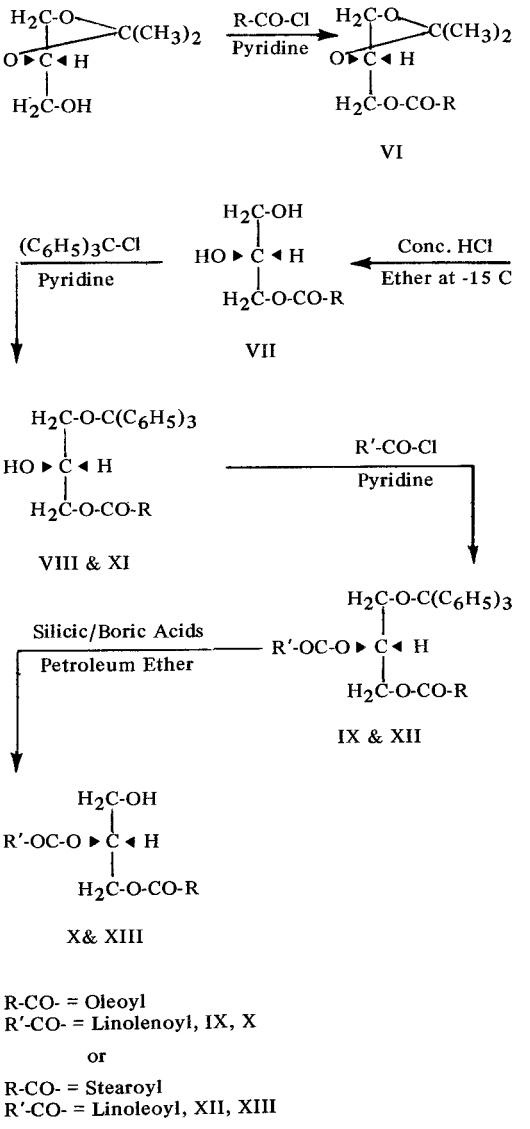
far to the author's knowledge, no enantiomeric, mixed acid, saturated and polyunsaturated, 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols have been synthesized.

Now a general procedure is reported that permits the synthesis of mixed acid, saturated and polyunsaturated, 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols using D-mannitol as a starting material.

The synthesis of 1-stearoyl-2-linoleoyl-*sn*-glycerol, 1-stearoyl-2-linolenoyl-*sn*-glycerol starts with 1-stearoyl-*sn*-glycerol, and the synthesis of 2-linoleoyl-3-stearoyl-*sn*-glycerol and 2-linolenoyl-3-oleoyl-*sn*-glycerol starts with 3-stearoyl-*sn*-glycerol and 3-oleoyl-*sn*-glycerol, respectively.

1-Stearoyl-*sn*-glycerol was obtained by two different synthetic pathways: (a) from D-mannitol by a more complicated procedure (12) as in Figure 1, and (b) by a relatively simple procedure using the same sequence of reactions as illustrated in Figure 2, but starting from L-mannitol (14).

The synthesis of 1-stearoyl-2-linoleoyl-*sn*-glycerol and 1-stearoyl-2-linolenoyl-*sn*-glycerol is illustrated in the reaction scheme, Figure 1, and the synthesis of 2-linoleoyl-3-stearoyl-*sn*-glycerol and 2-linolenoyl-3-oleoyl-*sn*-glycerol is illustrated in the reaction scheme, Figure 2.



R-CO- = Oleoyl
R'-CO- = Linolenoyl, IX, X

or

R-CO- = Stearoyl
R'-CO- = Linoleoyl, XII, XIII

FIG. 2. Synthesis of 2,3-diacyl-*sn*-glycerol from D-mannitol via above intermediates.

EXPERIMENTAL PROCEDURES

Materials

L-mannitol was prepared from quebracitol via L-inositol. (The method for the preparation of L-mannitol (15,16,17) has been simplified and adapted to the preparation of larger amounts. The details of preparation will be reported elsewhere.) D-mannitol (certified) was obtained from Fisher Scientific Company. Triphenylmethyl chloride was prepared by methods of Gomberg (18,19) as described by Bachmann (20). For further purification the triphenylmethyl chloride was distilled in vacuo, bp 155 C/0.08 mm Hg; mp 113-114 C. Linoleic acid and linolenic acid of high purity were obtained from the Hormel Institute, University of Minnesota. Oleic acid of a purity of at least 99.8% was prepared according to the method of Rubin and Paisley (21). Stearic acid, 99.5% pure, was obtained from Fluka A.G. Buchs SG, Switzerland. All fatty acids were converted into the corresponding chlorides with oxalyl chloride. Anhydrous pyridine was prepared from "Certified," infrared analyzed pyridine. The benzene was thiophene free and dried over sodium wire. The silicic acid was Mallinckrodt, 100 mesh (powder), "Analytical Reagent" with a 12% loss in weight on ignition. Boric acid was certified Fisher Scientific Company Stock. All experiments with unsaturated fatty acids were carried out under a nitrogen atmosphere.

Preparation of 1-Stearoyl-3-O-Triphenylmethyl-*sn*-Glycerol (I)

1-Stearoyl-*sn*-glycerol (25.0 g, 0.07 mole) was dissolved in 200 ml of a mixture of anhydrous pyridine and anhydrous benzene (1:1 v/v). A solution of 19.5 g (0.07 mole) of pure triphenylmethyl chloride in 50 ml of anhydrous benzene was added with stirring under anhydrous conditions. The reaction mix-

TABLE I

Analytical Data and Yields of the Acyl-Isopropylidene-*sn*-Glycerol (VI), Monoacyl-*sn*-Glycerol (VII), Monoacyl-Monotriphenylmethyl-*sn*-Glycerols (I, VIII, IX), Diacyl-Monotriphenylmethyl-*sn*-Glycerols (II, IV, IX, XII) and Diacyl-*sn*-Glycerols (III, V, X, XIII)

Compounds	Formula and mol wt	Per cent carbon		Per cent hydrogen		Iodine value		Per cent yield
		calc.	found	calc.	found	calc.	found	
I	$C_{40}H_{56}O_4$ (601)	79.95	79.99	9.40	9.47	---	---	73
II	$C_{58}H_{87}O_5$ (864)	80.60	81.00	10.14	10.21	58.7	59.0	95
III	$C_{39}H_{72}O_5$ (621)	75.42	75.33	11.70	11.57	81.8	82.1	95
IV	$C_{58}H_{85}O_5$ (862)	80.78	80.91	9.93	9.89	88.3	88.7	95
V	$C_{39}H_{70}O_5$ (619)	75.67	75.55	11.40	11.42	123.0	122.5	76
VI	$C_{24}H_{44}O_4$ (296)	72.68	72.62	11.20	11.16	64.0	63.8	96
VII	$C_{21}H_{40}O_4$ (357)	70.08	69.98	11.87	12.00	71.2	71.2	92
VIII	$C_{40}H_{54}O_4$ (599)	80.20	80.15	9.10	9.23	42.4	42.5	80
IX	$C_{58}H_{83}O_5$ (860)	80.97	81.01	9.72	9.68	118.1	119.0	95
X	$C_{39}H_{68}O_5$ (617)	75.95	76.00	11.12	11.21	164.6	165.0	56
XI	$C_{40}H_{56}O_4$ (601)	79.95	79.92	9.40	9.50	---	---	95
XII	$C_{58}H_{87}O_5$ (864)	80.60	80.35	10.14	10.18	58.7	58.9	93
XIII	$C_{39}H_{72}O_5$ (621)	75.42	75.50	11.70	11.58	81.8	82.0	75

ture was kept at 45 C for 24 hr and then the reaction product, 1-stearoyl-3-O-triphenylmethyl-*sn*-glycerol was isolated and purified as follows: The reaction mixture was diluted with 300 ml of diethyl ether and the mixture was washed in succession with a 300 ml portion of distilled water, two 300 ml portions of ice cold 2 N sulfuric acid, one 300 ml portion of distilled water, two 300 ml portions of a saturated sodium bicarbonate solution, and finally with two 300 ml portions of distilled water. The solution was dried with 100 g of anhydrous sodium sulfate, and the solvents were removed by distillation under reduced pressure from a bath at 35 C. The remaining material was kept in vacuo at 0.08 mm Hg until its weight was constant. The 1-stearoyl-3-O-triphenylmethyl-*sn*-glycerol weighed 41.0 g, and was shown to be a fairly homogeneous substance by thin layer chromatography. Two recrystallizations from petroleum ether (bp 30-60 C) at +6 C yielded a chromatographically pure material.

Preparation of 1-Stearoyl-2-Linoleoyl-3-O-Triphenylmethyl-*sn*-Glycerol (II)

To a solution of freshly prepared 1-stearoyl-3-O-triphenylmethyl-*sn*-glycerol (I), 18.0 g (0.03 mole) in 60 ml of anhydrous benzene and 5 ml of anhydrous pyridine was added freshly prepared and distilled linoleoyl chloride, 9.0 g (0.03 mole) in 20 ml of anhydrous benzene. The reaction mixture was kept under anhydrous conditions at 40 C for 24 hr, and then diluted with 200 ml of diethyl ether. The mixture was washed in succession with a 250 ml portion of distilled water, with two 250 ml portions of ice cold 2 N sulfuric acid, two 250 ml portions of saturated sodium bicarbonate solution and finally two 250 ml portions of distilled water. The solution was dried with 50 g of anhydrous sodium sulfate and the solvents were evaporated under reduced pressure. The remaining oil was dissolved in 200 ml of petroleum ether (bp 30-60 C) and the solution was kept for 20 hr at -6 C. The turbidity was removed by centrifugation. The clear supernatant was decanted and again concentrated under reduced pressure. The concentrated product was kept in vacuo of 0.05 mm Hg until its weight was constant. The 1-stearoyl-2-linoleoyl-3-O-triphenylmethyl-*sn*-glycerol, a viscous oil, weighed 24.5 g.

The material without further purification exhibited a specific rotation of $[\alpha]_D + 12.2°$ in chloroform *c*,10 and an iodine value of 59.0, calculated 58.7. Thin layer chromatography showed a quite homogeneous material.

To obtain analytically pure material, 1-stearoyl-2-linoleoyl-3-O-triphenylmethyl-*sn*-glycerol was chromatographed on silicic acid, although it involves partial detritylation. A solution of 23 g of material dissolved in 100 ml of benzene (U.S.P., redistilled) was passed through a 300 g silicic acid column, 3.5 cm wide and 50 cm long. The column was eluted with benzene until eluate was free of solute. In the initial benzene eluate pure 1-stearoyl-2-linoleoyl-3-O-triphenylmethyl-*sn*-glycerol was recovered corresponding to about 70% of the material applied to the column. In the later benzene eluate triphenylcarbinol appeared in small amounts. The benzene-diethyl ether mixture (4:1 v/v) eluted the detritylated product, 1-stearoyl-2-linoleoyl-*sn*-glycerol (5 g) with about 30% yield.

Preparation of 1-Stearoyl-2-Linoleoyl-*sn*-Glycerol (III)

The removal of the triphenylmethyl protec-

TABLE II

Physical Properties of the Acyl-Isopropylidene-*sn*-Glycerol, Monoacyl-*sn*-Glycerol, Monoacyl-Monotriphenylmethyl-*sn*-Glycerols, Diacyl-Monotriphenylmethyl-*sn*-Glycerols and Diacyl-*sn*-Glycerols

Compounds	Specific rotation in chloroform c,10, deg	Refractive index at 25 C	Density at 20 C	Physical state at 20 C
I	+ 3.85	---	----	Crystalline
II	+12.50	---	---	Oil
III	- 2.80	1.4710	0.9230	Oil
IV	+12.40	1.5125	---	Oil
V	- 2.70	1.4702	0.9315	Oil
VI	+ 4.80	1.4560	0.9109	Oil
VII	-- 3.60[a]	---	---	Paste
VIII	- 3.60	---	---	Oil
IX	12.00	1.5240	---	Oil
X	+ 2.60	1.4800	0.9318	Oil
XI	- 3.70	---	---	Crystalline
XII	-12.30	---	---	Oil
XIII	+ 2.70	1.4710	0.9230	Oil

[a]Specific rotation measured in pyridine c,10. Reported for 3-stearoyl-*sn*-glycerol -3.58 deg, in pyridine c,12.3(14).

tive group on a silicic acid column varied from one diacyl-*sn*-glycerol to another, and also from one batch of silicic acid to the other. However later work showed that silicic acid containing 10% by weight of boric acid offers an excellent mixture for the complete removal of triphenylmethyl protective group from any diacyl-O-triphenylmethyl-*sn*-glycerol.

The 1-stearoyl-2-linoleoyl-3-O-triphenylmethyl-*sn*-glycerol (14 g) was dissolved in 150 ml of petroleum ether (bp 30-60 C) and the solution was passed through a column of 300 g of a freshly prepared silicic acid-boric acid mixture (10:1 w/w). (The experimental preparation and application of silicic acid-boric acid mixture for the removal of triphenylmethyl protective groups is explained in a separate paper, now in preparation.)

The column was 3.5 cm wide and 60 cm long, and was eluted with petroleum ether, petroleum ether-diethyl ether (96:4 v/v) and petroleum ether diethyl ether (90:10 v/v). The petroleum ether fraction eluted only very small amounts of original material. Triphenylcarbonol was eluted with petroleum ether-diethyl ether mixture (96:4 v/v), and the detritylated product, 1-stearoyl-2-linoleoyl-*sn*-glycerol was recovered in the petroleum ether-diethyl ether mixture (90:10 v/v) in about a 95% yield. 1-Stearoyl-2-linoleoyl-*sn*-glycerol is an oil at room temperature and solidified gradually at +6 C.

1-Stearoyl-2-Linolenoyl-3-O-Triphenylmethyl-*sn*-Glycerol (IV), and 1-Stearoyl-2-Linolenoyl-*sn*-Glycerol (V)

These were prepared by the same procedure as II and III with proper choice of acylating reagent.

Preparation of 1,2-Isopropylidene-3-Oleoyl-*sn*-Glycerol (VI)

The method for the synthesis of saturated 3-acyl-*sn*-glycerols by Baer and Fischer (14) was adapted to the synthesis of 3-oleoyl-*sn*-glycerol. To a solution of freshly prepared 1,2-isopropylidene-*sn*-glycerol 6.8 g (0.05 mole) and 5 ml of anhydrous pyridine in 50 ml of anhydrous benzene was added 15.0 g (0.05 mole) of freshly prepared and distilled oleoyl chloride dissolved in 50 ml of anhydrous benzene. The reaction mixture was kept for 24 hr at room temperature, diluted with 150 ml of diethyl ether, and then freed of pyridine with ice cold 1 N sulfuric acid, then with saturated sodium bicarbonate solution, and finally with water. After drying the diethyl ether-benzene layer with anhydrous sodium sulfate the solvents were evaporated under reduced pressure to give 18.8 g of 1,2-isopropylidene-3-oleoyl-*sn*-glycerol.

Preparation of 3-Oleoyl-*sn*-Glycerol (VII)

To a -15 C solution of 1,2-isopropylidene-3-oleoyl-*sn*-glycerol in 100 ml of diethyl ether was added 100 ml of -15 C concentrated hydrochloric acid. The reaction mixture was stirred for 15 min and then diluted with 800 ml of ice cold distilled water. The reaction mixture was allowed to stand with occasional stirring for 20 min in a bath of -15 C ice-salt mixture. The 3-oleoyl-*sn*-glycerol was then extracted three times with 300 ml portions of diethyl ether. The combined diethyl ether extracts after washing with 300 ml of ice cold distilled

water were dried over 100 g of anhydrous sodium sulfate. The solvent was then removed by distillation under reduced pressure at 30 C bath temperature. The remaining material was kept in vacuo at 0.05 Hg until the weight was constant. The 3-oleoyl-*sn*-glycerol (weight 15.5 g) was used in the next step without further purification.

The 3-oleoyl-*sn*-glycerol, a soft paste, was found to be soluble at room temperature in chloroform, diethyl ether, benzene and petroleum ether, and insoluble in water.

1-O-Triphenylmethyl-3-Oleoyl-*sn*-Glycerol (VIII), 1-O-Triphenylmethyl-2-Linolenoyl-3-Oleoyl-*sn*-Glycerol (IX) and 2-Linolenoyl-3-Oleoyl-*sn*-Glycerol (X)

These were prepared by the tritylation, acylation and cleavage procedures employed for compounds I, II and III.

Preparation of 1-O-Triphenylmethyl-3-Stearoyl-*sn*-Glycerol (XI)

The 2-linoleoyl-3-stearoyl-*sn*-glycerol was obtained by the same sequence of reactions as 2-linolenoyl-3-oleoyl-*sn*-glycerol from D-mannitol via 3-stearoyl-*sn*-glycerol (14). (See reaction scheme, Fig. 2).

1-O-Triphenylmethyl-2-Linoleoyl-3-Stearoyl-*sn*-Glycerol (XII) and 2-Linoleoyl-3-Stearoyl-*sn*-Glycerol (XIII)

These were prepared by the acylation and cleavage procedures employed for compounds II and III.

The removal of the triphenylmethyl protecting groups with silicic acid and boric acid mixture proceeded without acyl migration. All the detritylated products were examined on the TLC and no 1,3-diacyl-*sn*-glycerol could be detected on the chromatographic plates.

These diacyl-*sn*-glycerols containing polyunsaturated chains are sensitive to oxidation. Therefore they must be kept under a nitrogen atmosphere. To prevent the loss of optical activity they were stored under anhydrous conditions at -15 C.

All the analytical values, yields and the physical properties of the intermediates and of the 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols described in this paper are summarized in Tables I and II.

DISCUSSION

The chemical synthesis of optically active 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols containing two dissimilar fatty acid substituents, either a saturated in 1 position and a polyunsaturated in 2 position or monounsaturated in 3 position from D-mannitol and L-mannitol via 1-acyl-*sn*-glycerol and 3-acyl-*sn*-glycerol, respectively, follows a relatively simple procedure. However since L-mannitol is not commercially available it was deemed desirable to devise methods which permit the synthesis of mixed acid, saturated and polyunsaturated, 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols from commercially available D-mannitol.

The procedure developed by Pfeiffer et al. (13) cannot be adapted to the synthesis of mixed acid diacyl-*sn*-glycerols because the synthesis of mixed acid diacyl-*sn*-glycerols requires a step by step introduction of the two dissimilar fatty acid substituents (see reaction scheme, Fig. 1 and 2).

With these restrictions in mind, procedures have been developed that permit the synthesis of both enantiomeric forms of mixed acid, saturated and polyunsaturated, 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols, respectively, from D-mannitol.

Recent studies of the positional distribution of fatty acids in 1,2-diacyl-*sn*-glycerols of 3-phosphatidylcholine and 3-phosphatidylethanolamine by Lands and Hart (22), Brandt and Lands (23), and by Kuksis et al. (24-27) have indicated that the saturated fatty acids are in the 1 position, while all the polyunsaturated fatty acids are esterified in the 2 position of the glycerol moiety. This is also true for the nonrandom stereospecific distribution of fatty acid residues in the triacyl-*sn*-glycerols (28-31).

As most of the natural 1,2-diacyl-*sn*-glycerol structures contain saturated and a polyunsaturated fatty acid substituents, it seemed of interest to continue this work by synthesizing the following representatives: 1-Palmitoyl-2-oleoyl-, 1-palmitoyl-2-linoleoyl-, 1-palmitoyl-2-linoelnoyl- and 1-palmitoyl-2-arachidonoyl-*sn*-glycerols.

The synthesis of these 1,2-diacyl-*sn*-glycerols is now in progress in this laboratory.

ACKNOWLEDGMENT

The Medical Research Council of Canada supported this work.

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[Revised manuscript received March 3, 1971]