

and 23.7 tritium units in December, substantially below the groundwater ones. The latter reflect earlier inputs, perhaps from the preceding summer with 118 tritium units in May, 126.3 tritium units in June, 99.1 tritium units

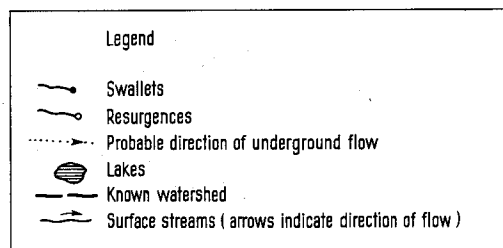
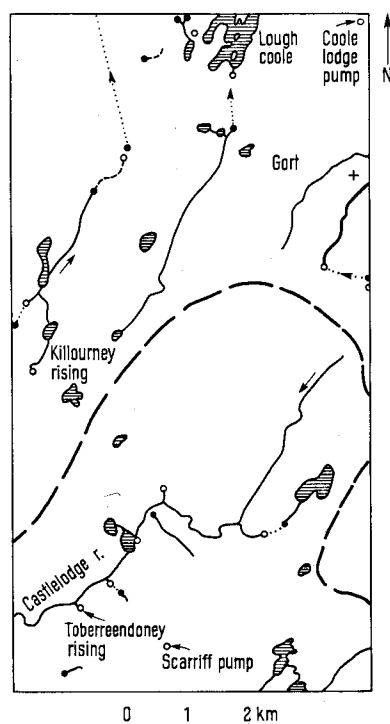
in July and 117.2 tritium units in August. This is consistent with karst studies showing that percolation water in the vadose zone passes to groundwater within a year. However, most summer rain is evapotranspired and also the limestone at the 4 sampling points is overlaid by calcareous boulder clay probably acting as a delaying mechanism. Consequently, a model employing a year with winter precipitation having tritium values of the right order of magnitude is proposed and this is 1966 where October had 98.04 tritium units and November 99.12 tritium units. Underground karstic interconnections exist and promote good mixing, thus eliminating one of the causes of groundwater stratification, namely, density stratification. Hence, the possible placing of the data into 2 groups (100.6, 92.2 and 79.2, 74.6) implies insufficient sampling rather than stratification. In summary, recent, i.e. late 1966, recharge is indicated with slow throughput and, referring to question 2 above, it is known to result from direct infiltration, at least in the cases considered here (although not in general throughout the Clare-Galway area). A stable isotope survey is now in progress⁸.

Zusammenfassung. Grundwasserproben aus der Gort-Ebene (West-Irland) wurden auf Tritium-Gehalt analysiert und die Ergebnisse mit dem bereits bekannten Tritium-Einstrom dieser Gegend verglichen. Registrierungen des «World Precipitation Network» in Wien ergaben den Nachweis eines offenbar langsamen Durchflusses in das Grundwasser-Aquifer-System.

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⁸ Acknowledgments. The authors are indebted to the Nuclear Physics Division, Atomic Energy Research Establishment, Harwell (England) for tritium analyses and to Mr. B. R. PAYNE, Section of Isotope Hydrology, IAEA, Wien (Austria) for providing unpublished tritium data from Valentia for the period 1966 to 1971.



Phosphatidyl-N-(2 Hydroxyethyl)-Alanine Synthesis of Distearoyl L- α -Glycerolphosphoryl-N-(2 Hydroxyethyl)-Alanine^{1,2}

KEMP and DAWSON³ reported in 1969 that they obtained evidence of a new phospholipid during their investigations of the Ciliatine containing phospholipids of rumen micro-organisms. This novel lipid gave N-(2 hydroxyethyl)-alanine as a product of hydrolysis.

The present communication describes the synthesis of the title compound V with the structure and configuration in which all the naturally occurring phospholipids occur. Stearic acid has been chosen as the fatty acid part, as this happens, more often than not, to be prevalent in the natural lipids.

DL-N-(2 hydroxyethyl)-alanine has been prepared according to the method of VIELES and SEGUIN⁴ with some modifications. The N-carbobenzoxy derivative of this amino acid has been prepared according to the method of BAER and PAVANARAM⁵. The product which gave correct

nitrogen values, without further purification, has been benzylated with an excess of benzyl chloride and triethylamine at 70°–75° and worked out as usual⁶.

¹ Dedicated to Professor Dr. ERICH BAER in honour of his 70th birthday.

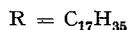
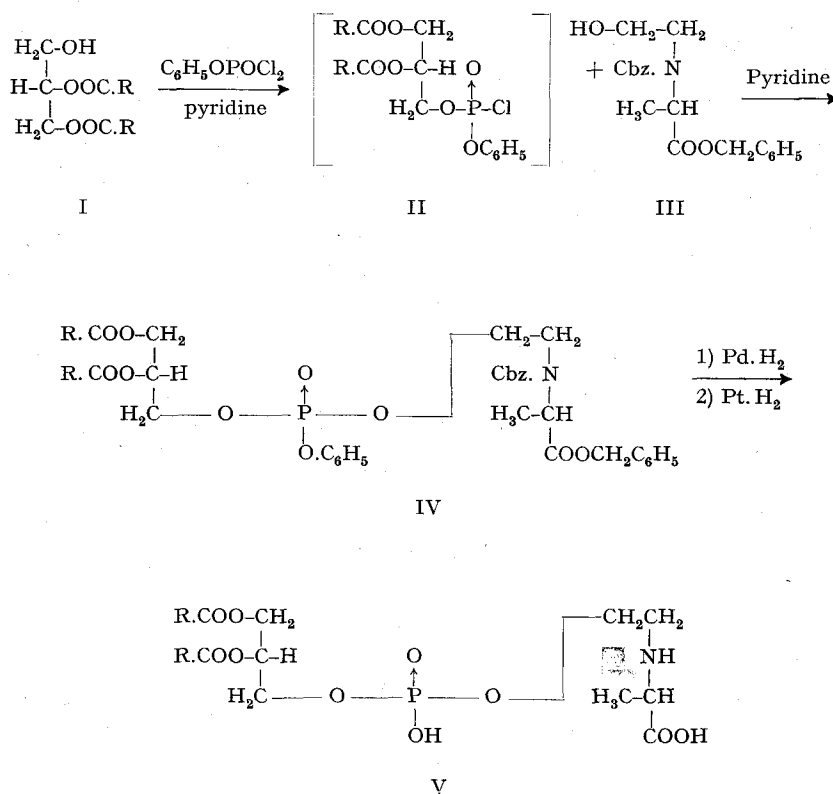
² The nomenclature used in this communication is that used by FISCHER and BAER for well-nigh 4 decades. However, according to the rules of IUPAC-IUB commission this should be 1,2-distearoyl-sn-glycero-3-phosphoryl-N-(2-hydroxyethyl)-alanine.

³ P. KEMP and R. M. C. DAWSON, *Biochim. biophys. Acta* **176**, 678 (1969).

⁴ P. VIELES and J. SÉGUIN, *C. r. Soc. Biol., Paris* **238**, 1819 (1954).

⁵ E. BAER and S. K. PAVANARAM, *J. biol. Chem.* **236**, 1270 (1961).

⁶ E. BAER, D. BUCHNEA and H. C. STANCER, *J. Am. chem. Soc.* **81**, 2166 (1959).



The oily product has been purified chromatographically on a silicic acid column. The benzylolester showed a single spot in TLC. Anal. calcd. for $C_{20}H_{23}O_5N$ Mol. Wt. 357.4; C 67.2; H 6.49; N 3.92; Found: C 67.15; H 6.56; N 3.85%.

O-(Distearoyl- α -glycerylphosphoryl-N-Carbonbenzoxy-(2-hydroxyethyl)-alanine was then prepared by phosphorylating *D*- $\alpha\beta$ -distearin (I) with monophenylphosphoryl dichloride and pyridine in dry chloroform and esterifying the phosphatidic acid monochloride (II) with N-Carbo-benzoxy-(2-hydroxyethyl)-alanine benzyl ester (III) in the presence of pyridine. The product on working up as usual⁵ and purifying by passing over a column of silicic acid gave a semi-solid which is pure in TLC. Anal. calcd. for $C_{65}H_{102}O_{12}NP$ Mol. Wt. 1120.5; C 69.65; H 9.17; N 1.25; P 2.76; Found: C 69.57; H 9.12; N 1.31, 1.30; P 2.56, 2.48%; $[\alpha]_D^{24} + 2.43^\circ$ (c 5.5 in dry chloroform).

The title compound V was then obtained by removing the protective groups from the intermediate IV by the consecutive catalytic hydrogenolysis using first palladium and then platinum as catalysts. The resulting white solid has been purified by passing over silicic acid and was eluted by chloroform containing 5% methanol. The pure (TLC single spot) phospholipid has been further crystallized from chloroform and methanol to yield white powder of m.p. 191°C . Anal. calcd. for $C_{44}H_{86}O_{10}NP$ Mol. Wt. 820.15; C 64.43; H 10.57; N 1.71; P 7.38%; Found (of two independent preparations): C 63.84, 64.01; H 10.56, 10.61; N 1.78, 1.75; P 3.38, 3.69%; $[\alpha]_D^{25} + 5.02^\circ$ (c 1.3 in dry chloroform)⁷.

In view of the pioneering efforts of SMEBY⁸ and subsequent work of PFEIFFER et al.⁹ and RAKHIT¹⁰ on the isolation of phospholipids from dog and hog kidney, the present synthetic phospholipid and its lyso derivative should provide interesting substrates for renin inhibitory activity.

As KEMP and DAWSON⁸ did not report any data of the natural compound, particularly the specific rotation, which is obviously due to the paucity of the lipid, the identification of our synthetic phospholipid with that of the naturally occurring one is still to be confirmed.

Zusammenfassung. Die erste Synthese eines Phosphatidyl-N-(2-hydroxyethyl)-alanins wird beschrieben.

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27 October 1971.*

⁷ The authors thank Dr. J. von EUW and Professor Dr. T. REICHSTEIN of the University of Basel (Switzerland) for kindly providing the rotation. As the optical purity is a very important criterion of the activity of the phospholipids; this has been determined on the Schmid and Heansch polarimeter (visual) as well as on the Perkin Elmer polarimeter (photoelectric) with excellent agreement.

⁸ R. SMEBY, S. SEN and M. F. BUMPUS, *Circulation Res.*, Suppl. 2, 20, 129 (1967). – R. SMEBY and F. M. BUMPUS, in *Kidney Hormones* (Ed. J. W. FISCHER; Academic Press Inc., New York 1971), p. 207.

⁹ F. R. PFEIFFER, *J. med. Chem.* 14, 493 (1971), 15, 58 (1912).

¹⁰ S. RAKHIT, *Can. J. Biochem.* 49, 1012 (1971).

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