61. E. Eagle, J. Am. Oil Chem. Soc., 37, 40 (1960).
62. V. P. Rzhekhin, Gossypol and Its Derivatives [in Russian], Leningrad (1955), p. 10.
63. Yu. P. Zalesov and A. L. Markman, Maslob. Zhir. Promst., 3, 8 (1960).
64. I. P. Nazarova, A. I. Glushenkova, and A. L. Markman, Khim. Prir. Soedin., 607 (1976).
65. A. L. Markman and V. P. Rzhekhin, Gossypol and Its Derivatives [in Russian], Moscow (1965), p. 194.
66. W. A. Pons and J. D. Guthrie, J. Am. Oil Chem. Soc., 26, 671 (1949).
67. W. A. Pons, C. L. Hoffpauir, and R. T. O'Connor, J. Am. Oil Chem. Soc., 27, 539 (1950).
68. C. M. Hall, L. E. Castillon, W. A. Guice, and C. H. Boatner, J. Am. Oil Chem. Soc., 25, 457 (1948).
69. R. W. Storherr and R. T. Holley, J. Agr. Food Chem., 2, 745 (1954).
70. M. S. Mathur, N. D. Sharma, and M. Sing, J. Food Sci. Technol., 9, 138 (1972).
71. N. P. Nazarova, G. A. Nezhinskaya, A. I. Glushenkova, and A. U. Umarov, Khim. Prir. Soedin., 608 (1979).
72. M. Z. Podol'skaya, Zh. Prikl. Khim., 17, 654 (1944).
73. A. L. Markman and S. N. Kolesov, Zh. Prikl. Khim., 28, 242 (1956).
74. S. N. Vil'kova and A. L. Markman, Zh. Prikl. Khim., 31, 1548 (1958).
75. S. P. Yun, V. A. Krakhmalëv, and D. E. Kharmats, Uzb. Biol. Zh., 24 (1979).
76. G. Scharm and J. H. Benedict, J. Am. Oil Chem. Soc., 30, 371 (1958).
77. P. K. Raju and C. M. Carter, J. Am. Oil Chem. Soc., 44, 456 (1967).
78. W. A. Pons, J. R. Pominski, W. H. King, J. A. Harris, and T. H. Hopper, J. Am. Oil Chem. Soc., 36, 328 (1959).
79. J. M. Dechary, J. Am. Oil Chem. Soc., 34, 597 (1957).
80. F. H. Mattson, J. B. Martin, and R. A. Volpenheim, J. Am. Oil Chem. Soc., 37, 390 (1960).
81. F. H. Smith, J. Am. Oil Chem. Soc., 35, 261 (1958).
82. W. A. Pons, C. L. Hoffpauir, and R. T. O'Connor, J. Am. Oil Chem. Soc., 27, 390 (1960).
83. W. A. Pons, R. T. Pittman, and C. L. Hoffpauir, J. Am. Oil Chem. Soc., 35, 93 (1958).
84. W. A. Pons, S. L. Hoffpauir, and R. T. O'Connor, J. Am. Oil Chem. Soc., 28, 8 (1951).

THE SEARCH FOR PROSTAGLANDINS AND PROSTAGLANDIN-LIKE COMPOUNDS IN PLANTS

A. G. Panosyan

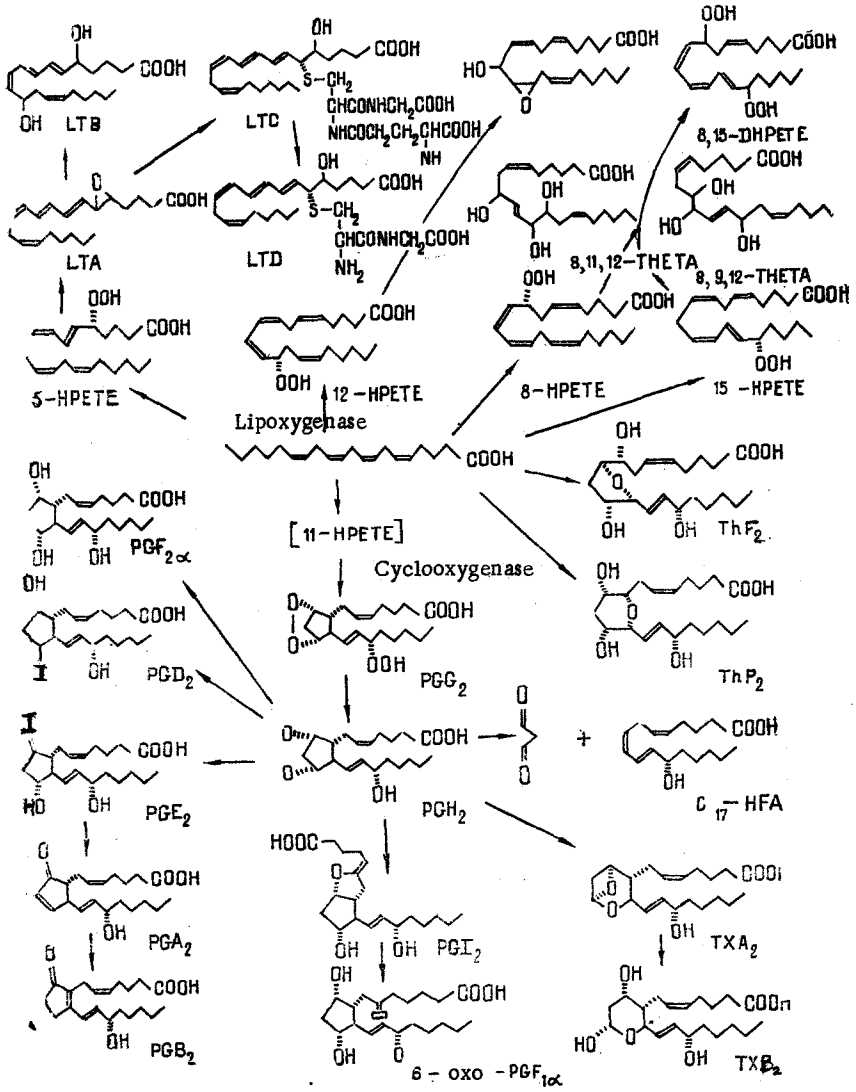
UDC 547.99:514.481:475.02+577.17

A review is given of the literature devoted to the question of the presence and distribution of prostaglandins and analogous products of the oxidative biotransformation of polyunsaturated fatty acids in plants. A comparison is made of the routes of oxidation of polyenic acids and the biological activity of the products formed in animal tissues and plants.

It was considered for a long time that the prostaglandins (PGs) are characteristic exclusively of mammals, where they are present in practically all tissues [1]. Then they were sought and rapidly found in many lower organisms [2]. PGs from the coral *Plexaura homomalla* [3] have even served as a raw material for chemical modifications [4] and for semi-industrial production, so high is their concentration in these organisms. There are reports of the formation and secretion of PGs and PG-like compounds by bacteria [5, 6]. As early as the beginning of the seventies, a search for PGs in plants had been begun but even today it is hardly possible reliably to state that the classical PGs are widely distributed in the vegetable kingdom.

A. L. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 139-146, March-April, 1981. Original article submitted July 17, 1980.

 0009-3130/81/1702-0102$07.50



Scheme 1. Pathways of the oxidation of arachidonic acid in animal tissues (the scheme does not include the mono- and dihydroxy acids formed from the corresponding peroxides).

The first attempt at screening PGs in plants was undertaken in 1971 [7]. Under the conditions usually used for isolating PGs from animal tissues, wheat bran yielded a compound (I) similar in its chromatographic behavior to $PGF_{2\alpha}$. However, it showed no biological activity in experiments on the contraction of isolated sections of the smooth musculature and on reduction with lithium tetrahydroaluminate the polymesitylate of the methyl ester of (I) formed octadec-9-en-1-ol. A detailed study of (I) showed that the compound isolated was 5,8,12-trihydroxyoctadeca-trans-9-enoic acid.

As the result of the screening of large number of plants with the aid of thin-layer chromatography (TLC), K. A. Attrep et al. [8] suggested that the yellow onion (*Allium cepa*) may be a source of PGA_1, which, as is well known, lowers the arterial pressure. They were also inspired by the fact that onion juice is used as a domestic remedy for hypertonia. After being washed with petroleum ether a centrifuged and acidified homogenate of the fresh plant was extracted with ethyl acetate. The crude extract so obtained lowered the blood pressure of rats and contained a substance with a similar R_f in TLC on silica gel to PGA_1, the spot being colored with a solution of copper acetate in phosphoric acid and also revealed in UV light. Column chromatography of the extract on silica gel led to the isolation of a fraction eluted together with an internal standard — $[^3H]PGA_1$. This fraction, like PGA_1, had an absorption maximum at 217 nm which, under the action of alkali, shifted to 270 nm, which is characteristic for the isomerization of PGA into PGB. The IR spectra

also proved to be similar, containing the absorption bands of a α,β-unsaturated cyclopentanone ring, on the basis of which the authors concluded that "although the determination of the exact structure of the fraction corresponding to PGA_1 is not conclusive for this study, these observations indicate that the probable structure of the fraction is very similar to PGA_1 or may be identical to it" [8]. The experiment described above was later repeated by the author of the present review, but the results presented were not confirmed.

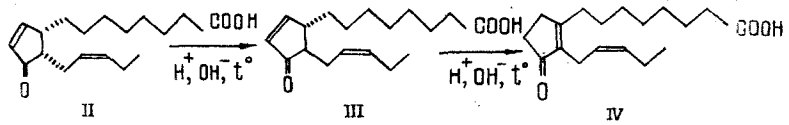
Similar facts are given in a report on the detection of PGs in the cambial zone of the Siberian larch (*Larix sibirica*) by Sh. T. Alaudinov and É. D. Levin (a comparison of TLC and of UV and IR spectra of a biologically active extract of the cambium with those of an extract of human seminal fluid) [9, 10].

A "pharmacological and chromatographic identification of prostaglandin-like compounds" in sugar-cane (*Saccharum officinarum*), bananas (*Musa paradisica*), and the coco palm (*Cocus nucifera* Lin.) was also reported by the Cuban scientists C. M. M. Cao and E. M. Cepero in 1975 at a conference on prostaglandins in Florence.

However, the only report on the investigation of the individual "mammalian PGs" PGE_2 and PGF_2 isolated from a plant extract and purified is a paper on the isolation from an aqueous extract of the red alga *Graciaria lichenoides* of a compound exhibiting a hypotensive action [12]. Judging from the method of isolation described, the authors did not set themselves the special task of finding PGs but simply isolated the active principle. An aqueous extract of the lyophilized organism was chromatographed successively on Amberlite XAD-2, Sephadex G-25, and octadecyl-silica gel (HPLC) with monitoring of the biological activities of the fractions. After the completion of the purification stage, two substances were obtained which were esterified with diazomethane, further purified by preparative TLC, and analyzed with the aid of TLC, ^{13}C NMR, ^1H NMR, ORD spectroscopy, and mass spectrometry (TMS derivatives). As a result, PGE_2 (the substance responsible for the hypotensive action of the plant) and PGF_2 were identified, their amounts being, respectively, 0.05-0.07% and 0.07-0.10% of the weight of the dry plant.

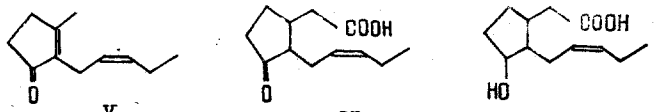
A somewhat different approach to this problem was made by B. Axelros et al. [13, 14], who studied the possibility of the biosynthesis of PGs by plant enzymes. [1-^{14}C]Arachidonic acid (AA) was incubated with the purified isoenzyme of soya beans — lipoxygenase-2, which catalyses the double dioxidation of arachidonic acid. The peroxide so formed was reduced with dithionite to the corresponding hydroxy derivatives. On a radiochromatogram of the reaction mixture, four zones containing radioactivity were detected: AA, 15-hydroxyeicosa-5,8,11,13-tetraenoic acid (15-HETE), 8,15-dihydroxyeicosa-5,9,11,13-tetraenoic acid (8,15-HETE), and $PGF_{2\alpha}$. The zone corresponding to the $PGF_{2\alpha}$ consisted of two components, which were analyzed by GLC-MS in the form of the trimethylsilyl (TMS) derivatives of the methyl esters and were identified as $PGF_{2\alpha}$ and 9,12-epoxy-8,11,15-trihydroxyeicosa-5,13-dienoic acid (ThF_2). The latter has been described previously by Pace-Asciak, who had studied the products of the oxidation of AA by the enzymes of ram seminal vesicles [15]. The main argument indicating the presence of a cyclopentane ring for the first of the peaks, coinciding in its retention time with the TMS derivative of the methyl ester of $PFG_{2\alpha}$, is the presence in the mass spectrum of an ion with m/e 217, $(CH_3)_3SiO{-}CH{=}CH{-}CH{=}\overset{+}{O}{-}Si(CH_3)$ which is considered characteristic for PGs of series F: However, it must be mentioned that for a definitive exclusion of alternative interpretations of the mass spectrum such as, for example, that of a trihydroxyeicosatrienoic acid it would be desirable to have available information on the number of double bonds in this compound either from the PMR spectrum of the mixture or from the mass spectra of the products of its hydrogenation. Such cyclization with the formation of analogs also takes place with eicosa-9,11,14-trienoic and γ-linolenic acids, but does not take place in the case of α-linolenic acid [14].

By an analogous incubation of α-linolenic acid with a acetone powder of grape seeds, D. Zimmerman et al. [16, 17] obtained three isomers (II-IV) of 8-[3-oxo-2-(pent-cis-2-enyl)-cyclopent-cis-4-enyl]octanoic acid or the 12-hydroxy derivative of the hypothetical phytonoic acid containing, like prostanoic acid, a cyclopentane ring.



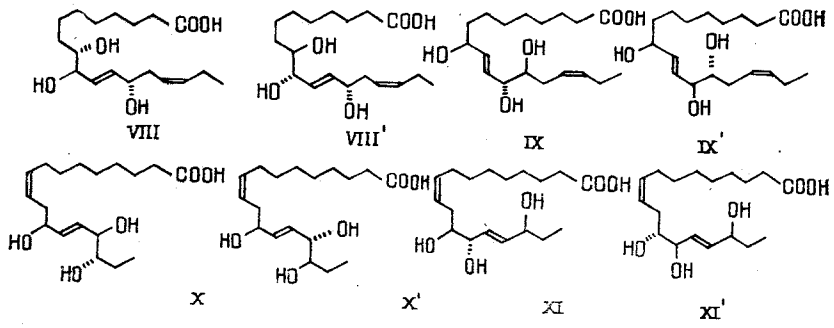
II III IV

The main component of the mixture was the cis isomer (II), which, under the action of acids or bases or as the result of thermal treatment is converted into the trans isomer (III) and into the tetrasubstituted α,β-unsaturated cyclopentanone isomer (IV) [17]. The enzyme preparation cyclizes other unsaturated fatty acids with 18, 20, and 22 carbon atoms and double bonds in the ω3,6, and 9 positions in a similar manner. In all cases, the ω6 hydroperoxide is an intermediate [18]. No information whatever is given about the biological activities of the compounds obtained nor have the absolute configurations of the asymmetric centers been established, but the authors, assuming that the above-mentioned compounds (II-IV) may be precursors of the plant growth inhibitors jasmone (V), jasmonic acid (VI), and cucurbic acid (VII) assume for the cis isomer (II) the same S,S configuration as in cucurbic acid.



V VI VII

The most purposeful search for PGs and their analogs is taking place among medicinal plants used by folk medicine precisely in those fields where the use of PGs is expected. This approach, consisting in the study of biologically active plants, has already proved itself with the case of red algae. On the basis of the same considerations, in the study of the roots of white bryony (*Bryonia alba* L.) a substance of acidic nature was extracted which exhibited prostaglandin-like activity on an isolated smooth-muscle organ in the presence of antagonists of acetylchloline, catecholamines, histamine, and serotonics [19]. The activity of the substance, like that of the prostagandins, fell in the presence of polyphloretin phosphate (a PG antagonist), but it was shown in concentrations one thousand times less than $PGF_{2\alpha}$, from which it did not differ in chromatographic mobility and in coloration by various detecting agents. Like some PGs [20 22], the substance increased the time of retraction of a blood clot [19] and showed a hypoglycemic action in doses of 5-10 γ/kg [23]. Although the substance migrated in the form of a homogeneous spot in TLC on silica gel in various systems of solvents, it was inhomogeneous and, as was found, consisted of an optically active fraction the main components of which were four diastereoisomeric pairs of biologically active trihydroxyoctadecadienoic acids (VIII-XI and VIII'-XI') which were separated satisfactorily only in the form of the TMS derivatives of the methyl esters in GLC-MS on a capillary column [24].



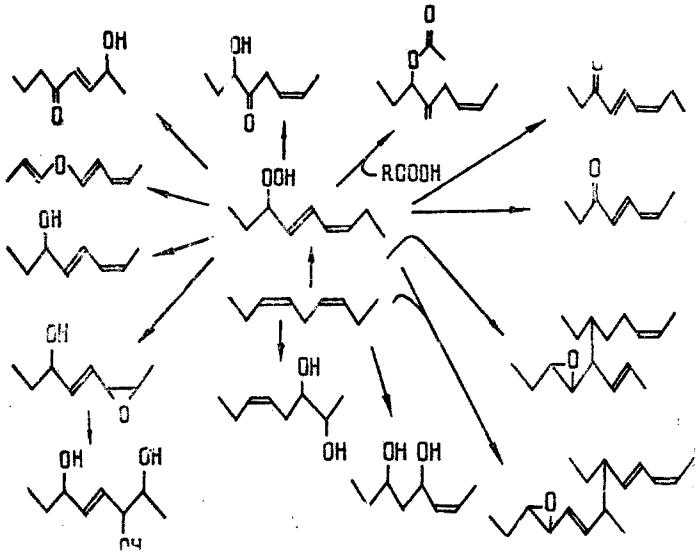
VIII VIII' IX IX'

X X' XI XI'

The C-20 homologs of the acids (VIII-XI and VIII'-XI') are formed in human thrombocytes from arachidonic and eicose-8,11,14-trienoic acids [25-27] (see Scheme 1). The oxidation of AA by the lipoxygenase route in the thrombocytes is intensified with high concentrations of the substrate and in the presence of glucose [27]. In patients with an inadequate lipoxygenase activity the plates aggregate at lower doses of AA and a tendency is observed in thrombogenesis [28]. The physiological role of the trihydroxy acids is still unclear, but is considered that plates aggregate only when two enzymes are active: a cyclooxygenase, which causes the first stage of aggregation, and a lipoxygenase which is necessary for the second stage [29]. The 8,15-dihydroperoxide (8,15-HPETE) formed when AA is incubated with soybean lipoxygenase-2 also effectively inhibits aggregation, while the hydroxy derivative 8,15-HETE is inactive [30]. In addition to thrombocytes, the lipoxygenase route of the oxidation of AA also takes place in white phagocyting cells and the lungs [31-36]. As a result, conjugated hydroxytrienic acids, called leukotrienes, and their thioesters with glutathione (LTC) and with cysteinylglycine (LTD), so-called slow reacting substances of anaphylaxis (SRS-A), are formed [31]. A number of the products of the lipoxygenase [34, 35] and cyclooxygenase [37-39] routes are mediators of the chemotaxis of leukocytes.

It is assumed that in a number of pathological deviations the hydroperoxides formed inhibit the biosynthesis of prostacyclin (PGI_2), whereupon the balance so necessary for the normal physiological state of the biosynthesis of PGI_2 and TXA_2 in the direction enhancing the action of TXA_2 is disturbed, and, as a result, depending on the cells in which this takes place, particular changes are observed: the aggregation of thrombocytes, bronchospasm, spasms of the blood vessels, and the development of atherosclerosis and diabetes [39].

A study of the physiological role of the lipoxygenase route for the oxidation of AA in processes of inflammation, cell migration, blood clotting, the secretion of mediators, and asphyxia in asthma and anaphylaxis and its connection with the cyclooxygenase route is passing through a period of vigorous development at the present time. It is just here, possibly, that natural homologs and analogs of the products of the lipoxygenase route of the oxidation of unsaturated fatty acids of plants will prove suitable and, perhaps, it is just this route which explains the healing properties of many medicinal plants the activity of which is due to unsaturated acids and the products of their oxidation. Scheme 2 shows the main routes of the enzymatic oxidation of unsaturated fatty acids in plants [40-42]. Scheme 3 illustrates the nonenzymatic routes of the autooxidation of unsaturated fatty acid, including the formation of PGG_2 and of PGH_2-like cyclic peroxides [41, 43, 44].

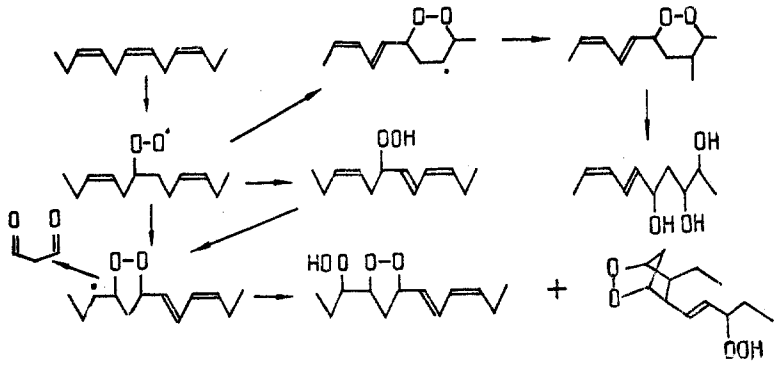


Scheme 2. Oxidation of unsaturated fatty acids by plant enzymes (the scheme does not include the simple prostanoic and phytonoic acids and tetrahydrofuran-containing acids described above).

The formation of the key and most active products of the cyclooxygenase route PGG_2, TXA_2, and PGI_2 in plants can be judged when their stable metabolites C_{17}-HFA, TXB_2, and 6-hydroxy-$PGF_{1\alpha}$, respectively, have been identified, which, in their turn are difficult to detect because of the absence of activity. The use of medicinal plants is, as a rule, accompanied by drying and not infrequently by the preparation of decoctions and infusions. The time of half-decomposition of PGG_2 in aqueous solutions is 5 min, and of TXA_2 30 sec at 37°C, while PGI_2 loses its activity on boiling in 15 sec or at 22° after 10 min at neutral pH values [39]. It is natural that it is $PHF_{2\alpha}$ that has the greatest chances of surviving under such conditions.

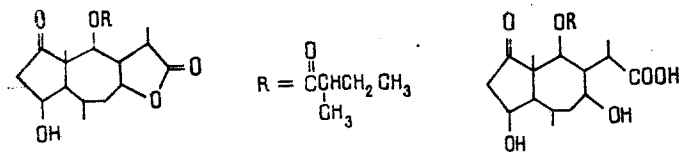
The screening of mixtures of substances of acidic nature isolated from a number of plants used as hypotensives, anti-inflammatory, bronchodilatory, hemostatic, and uterus-stimulating agents has shown that in some of them there are fractions of trihydroxydecenoic and/or trihydroxydecadienoic acids coinciding in R_f values with $PGF_{2\alpha}$ and exhibiting a weak prostaglandin-like activity [45]. These acids can be regarded as analogs of active synthetic secoprostaglandins [46].

However, a conclusion which appears more reliable is that these extracts contain other substances of acid nature differing in their R_f values from known PGs which are capable of causing the contraction of isolated smooth-muscle organs in the presence of indomethacin and



Scheme 3. Nonenzymatic oxidation of unsaturated fatty acids.

of antagonists of catecholamines, acetylcholine, histamine, and serotonin, i.e., capable of exciting PG receptors that have remained free. Not without grounds, it may be considered that a substance capable of acting on the PG receptors may be the known sesquiterpene lactone arnifolin (XII) (from *Arnica foliosa* and *Arnica montana*) [47], which tonizes the nuscular of the uterus and exhibits hemostatic properties [47].



XII XIII

The lactones are readily extracted from plants under the conditions for the isolation of PGs: extraction with buffer solutions having pH 8, acidification, and extraction of the aqueous phase with organic solvents. Under certain conditions, the lactone may open and appear in the form of a PGE analog (XIII). This hypothesis naturally requires checking but, at the same time, the isolation and study of compounds with prostaglandin-like properties present no less, and perhaps even greater, practical interest than the detection of unstable "mammalian PGs" showing no specificity. Such compounds may be regarded as preprepared biosynthetic analogs of PGs with an already known physiological action.

LITERATURE CITED

1. P. W. Ramwell, The Prostaglandins. Plenum Press, New York, Vol. 1 (1973).
2. T. Nomura and H. Ogata, Biochim. Biophys. Acta, 431, 127 (1976).
3. A. J. Weinheimer and R. L. Spraggins, Tetrahedron Lett., 5185 (1969).
4. G. L. Bundy, W. P. Schneider, F. H. Lincoln, and J. E. Pike, J. Am. Chem. Soc., 94, 2123 (1972).
5. E. Gulbis, M. Marion, J. Dumont, and E. Schall-Frederick, Prostaglandins, 18, 397 (1979).
6. L. Hellgren, B. Lindblom, J. Vincent, and L. Wilhelmsson, Experientia, 35, 1298 (1979).
7. P. W. Albro and L. Fishbein, Phytochemistry, 10, 631 (1971).
8. K. A. Attrep, J. M. Mariani, Jr., M. Attrep, Jr., Lipids, 8, 484 (1973).
9. É. D. Levin and Sh. T. Alaudinov, Khim. drevesiny, 101 (1978).
10. Sh. T. Alaudinov and É. D. Levin, Khim. drevesiny, 110 (1979).
11. C. M. M. Cao and E. M. Cepero, International Conference on Prostaglandins, Florence (1975), p. 77.
12. R. P. Gregson, J. F. Marwood, and R. J. Quinn, Tetrahedron Lett., 4505 (1979).
13. G. S. Bild, S. G. Bhat, C. S. Ramados, and B. Axelrod, J. Biol. Chem., 253, 21 (1978).
14. G. S. Bild, S. G. Bhat, C. S. Rhamadox, B. Axelrod, and Ch. S. Sweeley, Biochem. Biophys. Res. Commun., 81, 486 (1978).
15. C. Pace-Asciak, Biochemistry, 10, 3664 (1971).
16. D. C. Zimmerman and P. Feng, Lipids, 13, 313 (1978).
17. B. A. Vick, D. C. Zimmerman, and D. Weisleder, Lipids, 14, 734 (1979).
18. B. A. Vick and D. C. Zimmerman, Plant Physiol., 63, 490 (1979).

19. A. G. Panosyan, G. M. Avetisyan, V. A. Mnatsakanyan, T. A. Asatryan, S. A. Vartanyan, R. G. Boroyan, and S. G. Batrokov, Bioorg. Khim., 5, 242 (1979).
20. J. E. Kloeze, Experientia, 26, 307 (1970).
21. R. Bresler, M. Vargas-Cordon, and M. D. Lebovitz, Diabetes, 17, 617 (1968).
22. Thera Gesselschaft für Patentverwertung mbH, German Patent Application, 2,740,953 (1979); Chem. Abstr., 90, 210141f.
23. A. G. Panossian, G. M. Avetissian, V. A. Mnatsakanian, G. G. Gasparian, S. H. Pashitanian, G. S. Vartanian, and K. G. Karaguezian, Planta Med., 3, No. 3, 254 (1980).
24. A. G. Panossian, G. M. Avetissian, V. A. Mnatsakanian, S. G. Batrakov, S. H. Vartanian, E. A. Amrojan, and E. S. Gabrielian, Planta Med., in the press (1980).
25. P. Falardeau, M. Hamberg, and B. Samuelsson, Biochim. Biophys. Acta, 441, 193 (1976).
26. R. L. Jones, P. J. Kerry, N. L. Poyser, I. C. Walker, and N. H. Wilson, Prostaglandins, 16, 583 (1978).
27. R. W. Bryant and J. M. Bailey, Prostaglandins, 17, 9 (1979).
28. M. Okuma and H. Uchino, Food, 54, 1258 (1979).
29. C. E. Dutilh, E. Haddeman, G. H. Jouvenaz, F. Ten Hoor, and D. H. Nugteren, Lipids, 14, 241 (1979).
30. G. S. Bild, S. G. Bhat, B. Axelrod, and P. G. Latridis, Prostaglandins, 16, 795 (1978).
31. B. Samuelsson, Trends Pharm. Sci., 1, 227 (1980).
32. B. Samuelsson, S. Hammarström, and P. Borget, Adv. Inflam. Res., 1, 405 (1979).
33. J. J. Adcock, L. G. Garland, S. Moncada, and J. R. Vane, Prostaglandins, 16, 163 (1978).
34. S. R. Turner, J. A. Tainer, and W. S. Lynn, Nature (London), 257, 680 (1975).
35. E. Goetzle and R. R. Gorman, J. Immunol., 120, 526 (1978).
36. M. Riguad, J. Duzand, and J. C. Breton, Biochim. Biophys. Acta, 573, 408 (1979).
37. W. McClatchey and R. Synderman, Prostaglandins, 12, 415 (1976).
38. G. A. Higgs, E. McCall, and J. F. Youlten, Brit. J. Pharmacol., 53, 539 (1975).
39. S. Moncada and J. R. Vane, Pharm. Rev., 30, 293 (1978).
40. H. W. Gardner, Agric. Food Chem., 23, 129 (1975).
41. M. Scheutwinkel-Reich, H.-J. Stan, and Z. Lebensm. Unters. Forsch., 168, 32 (1979).
42. S. Husain, M. U. Ahmad, and S. M. Osman, Phytochemistry, 19, 75 (1980).
43. W. A. Pryor, J. P. Stanley, and E. B. Blair, Lipids, 11, 370 (1976).
44. E. N. Frankel, W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Garwood, and B. C. L. Weedon, Lipids, 12, 1055 (1977).
45. A. G. Panosyan, M. L. Barikyan, M. N. Lebedeva, E. A. Amroyan, and E. S. Gabrielyan, Khim. Prir. Soedin., 825 (1980).
46. J. B. Bicking, C. M. Robb, R. L. Smith, E. J. Cragoe, Jr., F. A. Kuehl, Jr., and L. R. Mendel, J. Med. Chem., 20, 35 (1977).
47. R. I. Evstratova, V. I. Sheichenko, K. S. Rybalko, and A. I. Ban'kovskii, The Search for New Biologically Active Substances [in Russian], VILR [All-Union Research Institute of Medicinal Plants], Moscow, No. 1 (1970), p. 148.