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KARL FREUDENBERG, BURCKHARDT HELFERICH, HERMANN O. L. FISCHER

A CENTENNIAL TRIBUTE

F. W. LICHTENTHALER
Institut für Organische Chemie, Technische Hochschule Darmstadt, D-6100 Darmstadt (F.R.G.)

In these fast-living times many scientific discoveries of past decades have become so commonplace that the basic achievements of their discoverers have sunk into oblivion or are grossly underestimated, despite all the appreciation they received. Pertinent examples are large portions of the prodigious contributions of *Karl Freudenberg, Burckhardt Helferich,* and *Hermann O. L. Fischer*, the three foremost chemists emerging from the Berlin school of Emil Fischer at the beginning of this century. Born in 1886, 1887, and 1888, respectively, they essentially share a hundredth anniversary which not only provides a unique opportunity to call to mind their importance in the advancement of carbohydrate chemistry and biochemistry, but also to trace the sources from which they derived their scientific thought and the impulses for their wide-ranging activities.

The fields these three great scientists penetrated in their extensive and persevering work over more than half a century were vast indeed, ranging from basic problems of organic stereochemistry to the chemistry of wood, from fundamental problems pertinent to mono- and poly-saccharides to key issues of enzymology. As a consequence, a brief, present-day summary of their accomplishments, presented here must necessarily be incomplete, fuller individual appreciations of their lives and works being given elsewhere[1–7], including bibliographies[4,7] of the close to 1000 publications that emanated from their laboratories. Nevertheless, an appraisal of their major achievements in carbohydrate chemistry, 20–30 years after the close of their active scientific careers, is considered of prime importance, not for learning interesting historical relationships or for keeping pivotal facts from oblivion, but for gaining a proper understanding and appreciation of the underlying creative and productive mechanisms, essential for anyone who is involved in the continuation of their work.

Freudenberg and Helferich commenced the study of chemistry at the universities of Bonn and Munich, respectively, which, however, obviously failed to satisfy the interests and ambitions of these keen chemistry students despite the presence of such renowned organic chemists as R. Anschütz and A. v. Baeyer. They changed in 1907 and 1909, respectively, to the Humboldt University of Berlin, where they encountered the fascinating personality of Emil Fischer, unapproachable to students but overwhelming in the brilliance of his lectures and in the scope of his scientific thought and activities. Under his direction, the Chemical Institute of the Berlin University has developed into one of the greatest scientific centers in

Europe. Experts in almost all fields of chemistry were working there, including such notables as Diels, Franz Fischer, Gabriel, Otto Hahn, Lise Meitner, Leuchs, and Traube. By 1908, Emil Fischer had largely relinquished work in the fields of carbohydrates, proteins, purines, and pyrimidines; he continued some work on sugars, but his main interest had turned to the chemistry of depsides and tannins. Accordingly, when Freudenberg was accepted as "Doktorand" in 1908, he was assigned a depside problem, comprising the synthesis of oligomeric *p*-hydroxybenzoic acid esters as tannin models. For this work he received his doctor's degree[8] in 1910 at the age of 24. Helferich, who had been allotted the theme of the glucosylation of higher alcohols for his dissertation[9], completed his doctoral studies a year later, likewise at the age of 24.

Emil Fischer's son Hermann had a slightly different entry into chemistry. He started his studies at Cambridge University in England in 1907, continued them in Berlin, and did his doctoral work at the University of Jena with Ludwig Knorr, at the behest of his father, because in another university where the young Fischer originally wanted to go, the habits in the Chemical Institute were found to be somewhat too alcoholic[5]. At that time Knorr was working on the separation of the keto and enol forms of β-diketones by crystallization at low temperature, and by application of this method Fischer succeeded in crystallizing the pure enol form of acetylacetone. Armed with his doctoral degree in 1912—also at the age of 24—he returned to Berlin to continue his research studies with his father, where he too was assigned a depside problem.

The period of 1912–1914, during which the three young chemists did postdoctoral work with Emil Fischer, turned out to be a highly productive phase of scientific collaboration. Freudenberg forcefully pushed ahead with synthetic investigations on the tannins[10], H. O. L. Fischer employed the methoxycarbonyl group for the protection of phenolic hydroxyl groups, which lead to syntheses of the *ortho*- and *para*-orsellinic acids[11], and Helferich, in continuation of his glycosylation studies, pioneered the synthesis of the first purine nucleoside[12]. Apart from the impressive series of high quality publications[10–12], this time of association with their illustrious teacher left its distinctive mark on the three congenial scientists, laying the foundations for the work they were to do later on their own, a fact that each of them was aware of and, in later years, repeatedly referred to. Helferich, in 1951, expressed this in the words "First and foremost I owe thanks to Emil Fischer through whom I was led to whatever I could achieve"[13], or, in the perception of Freudenberg in 1962: "Teamwork, as Emil Fischer developed it, demands a superior leader. It was magnificent to work under his direction and yet independently; as a research instructor, he blazed the trail for the generations succeeding him"[14].

This creative and prolific period in the development of these highly promising young scientists was rudely terminated by the outbreak of World War I, for each was drafted into the German Army at the beginning and had to serve, on the front in France mainly, for the ensuing four and one-half years. Emil Fischer under these adverse circumstances strived to keep the Chemical Institute in Berlin functioning

as well as possible, but could not prevent it from becoming devastatingly empty towards the end of the war. When that catastrophe finally ceased, it not only marked the end of German preeminence in organic chemistry that had prevailed for half a century, but with the death of Emil Fischer in July 1919 it also marked the end of the stellar career of one of the greatest organic chemists of this century, comparable in scope only to that of Liebig and Kekulé in the last.

Thus, in 1919, when a return to academic research became possible for each of the three, "it was a great effort to make up for the lost years, relearn all that we had forgotten, and bring ourselves up to date in the sciences"[5], a task that was made all the more difficult by the precarious political situation and the economic turmoil pervading the country. However each man, in his own particular way, managed to successfully redevelop his career in chemistry.

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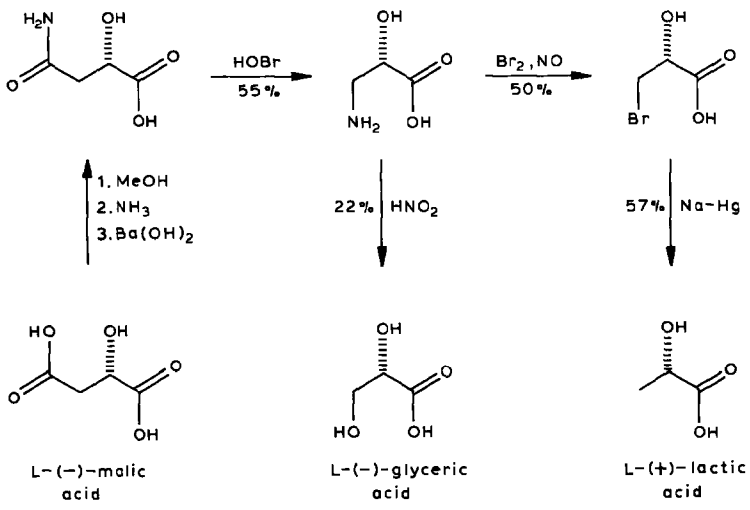
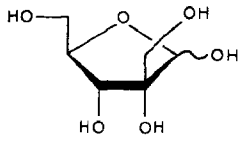
Evidence of drive and self-confidence was given by the young Freudenberg in that he managed to start work on his own while still with E. Fischer, and, maybe even more characteristically, that he effected a disengagement from his illustrious teacher on his own decision. As a first independent research topic he had chosen to study the configurational relationships between tartaric, malic, lactic, and glyceric acids (1914, ref. 15), accomplishing the conversion of levorotatory malic acid *via* a series of reactions, noteworthy for the time, into the equally levorotatory glyceric and lactic acids (see Scheme). When, prior to publication, he presented the results at an institute colloquium, apparently with success, the renowned physicist Madelung overwhelmed him with unstinted praise saying: "Das war eine echte Fischer-Arbeit". Freudenberg's reaction was one of painful awakenings — "If somebody would have hit me on my head with a truncheon, the effect would have



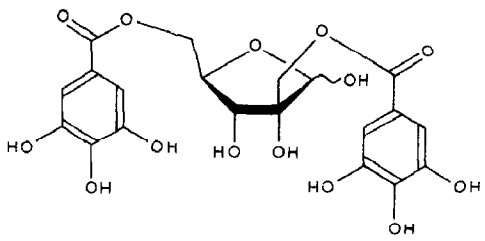
been the same; what Madelung said was true, alarmingly true, and in a flash I realized to what extent I was caught in the world of ideas of my teacher"[2]. His immediate response was to disengage from Emil Fischer by accepting an assistantship at the University of Kiel a few days later. There, he was able to complete his habilitation in the fall of 1914, just before military service in World War I interrupted all scientific activities for four years and brought his scientific career to a temporary halt. In 1919 he resumed his position at Kiel, which was followed in rapid succession by professorships in Munich (1920), Freiburg (1921), Karlsruhe (1922), and finally in Heidelberg (1926) as the successor to Curtius.

Three major lines of research, of which the roots may be clearly traced back to his time with Emil Fischer, were taken up in the early twenties and, growing in scope and intensity, carried on through the nearly five decades of his scientific career. These were: (i) investigations on the absolute configuration of sterically related compounds, (ii) studies on mono- and poly-saccharides, and the further exploration of the chemistry of the tannins. Along the latter theme, his (iii) work on witch hazel tannin uncovered the first branched-chain sugar, hamamelose (**1**), of which it could be shown[16], with the rather modest means for structural elucidation available then, that a hydroxymethyl branch is present next to the aldehyde group, and that the actual hamameli-tannin is its digalloyl ester **2**; only the configuration of **1** remained to be established later[17]. Basic work on other gallotannins, *e.g.* the far more complex chebulinic acid[18,19], followed, as well as extensive investigations on catechin-derived and other flavonoid tannins[20], which form the solid basis of our present knowledge in this field.



Hamamelose

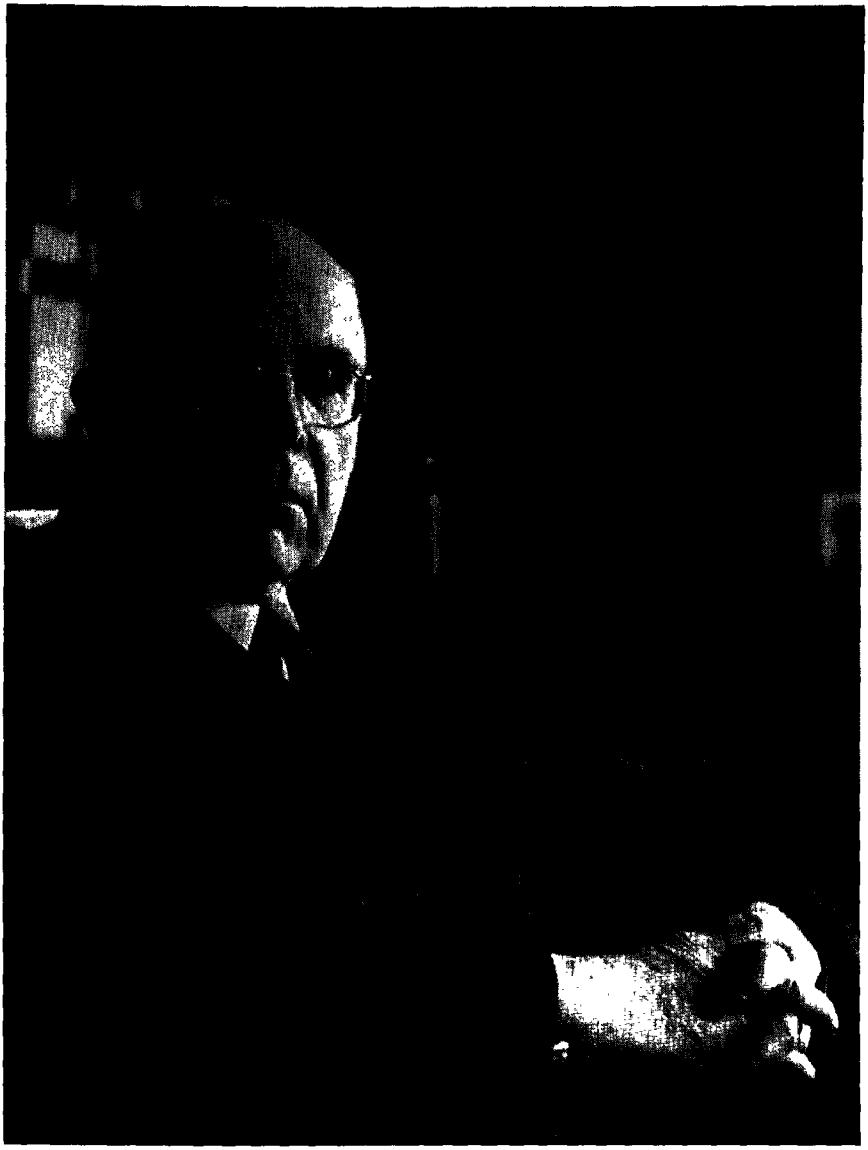
1



Hamamelitannin

2

Keenly interested in the general problems of stereochemistry, Freudenberg extended his Berlin work[15] on "series of sterically related compounds" to the available α-hydroxy, α-halo, and α-amino acids[21], all then being of unknown absolute configuration; one notable example from these studies[22] was the conversion of L-lactic acid into L-alanine in 1925. Information on the configuration of these compounds was derived from rotational data, yet, characteristically, not from the sign and absolute values for the individual compounds, but from the direction of the rotational changes produced when they were chemically derivatized in the same manner. As more material accumulated, these observations matured into the "optical shift rule"[23] (Optischer Verschiebungssatz), this having distinct



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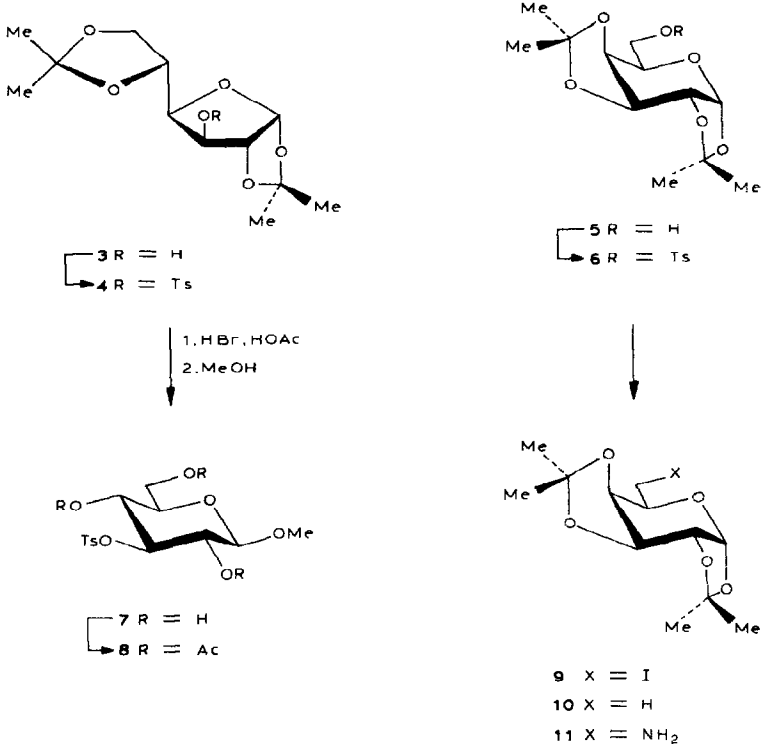
repercussions on the application of Hudson's isorotation rules to the sugars. Despite its limitations, this simple Freudenberg rule proved to be one of the major early tools for unravelling the absolute configurations of compounds with one or two centers of asymmetry. A further major outgrowth of his intense preoccupation with stereochemistry was the appearance, in 1933, of the famous 1500-page treatise "Stereochemie"[24], initiated, edited, and partly written by Freudenberg, which was to become *the* reference book on the subject, paving the way for all future developments.

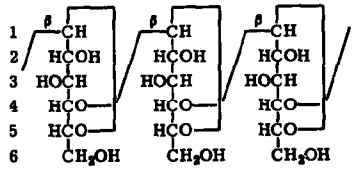
Into this first period of prodigious activity also falls the main part of Freudenberg's contributions to the chemistry of monosaccharides as well as of cellulose and starch. Starting in 1922 (ref. 25), the structures of the di-*O*-isopropylidene derivatives of the common sugars, namely, "diacetone-glucose (**3**), -galactose (**5**), -mannose, and -xylose", originally discovered by Emil Fischer, were unequivocally ascertained. Of the numerous derivatives prepared, one type was to attain particular importance, namely the sulfonic acid esters, the 3-*O*-tosyl-D-glucose derivative **4** and the 6-*O*-tosyl-D-galactose **6** in fact being the first ever prepared[25]. Various aspects of their remarkably interesting properties and potentialities gradually evolved, such as the displacement with iodine[26] (*e.g.* **6**→**9**), with ammonia (**6**→**11**) and hydrazine[25], or their reduction[26], opening up a huge territory by making possible the preparation of hitherto inaccessible deoxy-, halo-, and amino-sugars[27].



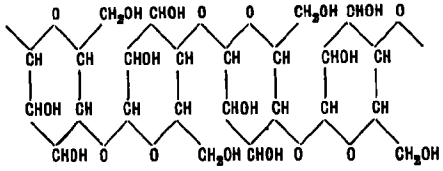
Other notable results from this period are the synthesis of a series of di- and tri-saccharides, albeit *via* the somewhat inadequate classical Koenigs–Knorr procedure[28], and the clarification of the anomalous behaviour of certain acetohalogeno sugars on methanolysis, namely the formation of orthoesters[29], not previously observed. Another topic being worked on in the early thirties was the specificity factors of blood groups A and B. Whilst it had been suggested at that time that the blood groups are characterized by certain glycoproteins on the erythrocyte membrane, nothing was known of the nature of the sugar moieties involved or the sequence of the sugars in the putative oligosaccharides. Thus, it is all the more surprising that Freudenberg was able to characterize the carbohydrate composition of blood group A substance to the extent of finding that galactose and N-acetylglucosamine are present in these glycoproteins[30].

Simultaneously with the above studies, polysaccharides were being investigated, a group of biopolymers that in the early twenties "was veiled in mystical darkness"[1] and, as a consequence, open to such precarious speculation as even seriously calling into question the high-molecular nature of cellulose and starch. Freudenberg had already tackled the cellulose structural problem in 1921, concluding, on the basis of the yield of octaacetylcellobiose obtained, that cellulose is composed of cellobiose units at least to the extent of 60%, and, most probably, totally[31]. In the ensuing years the experimentally elaborated and other available data accumulated consolidated so far, that in May of 1928, the valid structure of cellulose could be published[32], followed, only a month later, by an almost identical structural proposition by W. N. Haworth[33]. A detailed account of this development was given, 40 years later, by Freudenberg himself[34]—a fascinating piece of reading.

Structural Proposals for Cellulose



Freudenberg (May 1928)[32]

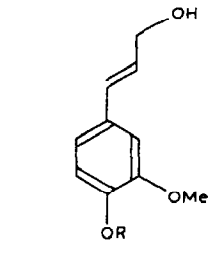


Haworth (June 1928)[33]

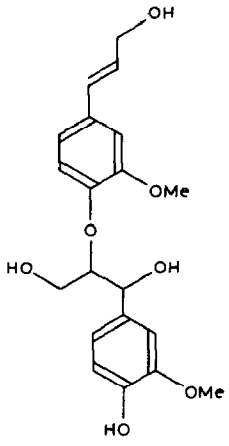
Equally important contributions to our concepts of starch structure emanated from Freudenberg's laboratory, such as a meticulous study of the kinetics of starch hydrolysis[35] and the characterization of the degradation products obtained after methylation[36]. The recognition that the Schardinger dextrins are not basic building blocks of starch but cyclic products formed by enzymatic degradation[37] led to the concept of a helical arrangement for amylose, with a central channel of about the

same diameter as in α-cyclodextrin. In this channel, as in the inclusion compounds characterized[38], iodine is sequestered, thus intelligibly explaining the blue iodine reaction[39]. As early as 1939 already Freudenberg had stated[37]: "One should return in this field too to the concept of screw-like arrangements, in which the major emphasis lies on hydrogen bonds, for it is to be assumed that the principle of order represented in protein chemistry also prevails in starch". This suggestion in fact proved to be the first proposal of a helical structure for a macromolecule.

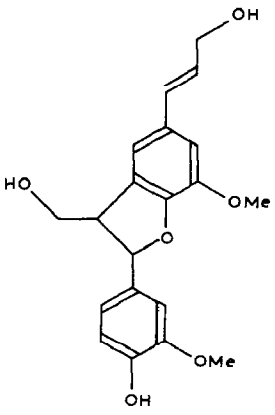
The immense burden of the political situation in Germany in the thirties, with which Freudenberg could not identify—particularly when colleagues and co-workers were dismissed due to their non-Aryan origins—increasingly impeded productive scientific work, which became essentially nonexistent during the last years



12 R = H
13 R = β-D-glucosyl



Guaiacylglycerol coniferyl ether
14



Dehydroconiferyl alcohol
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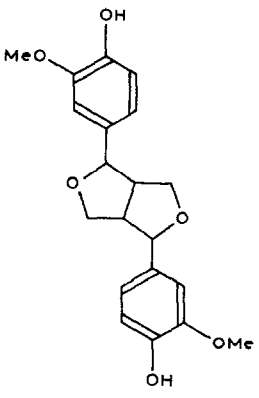

Pinoresinol
16

of the war. The end of the period, to Freudenberg, was like an awakening from a nightmare. Although nearly 60 already, and despite the huge difficulties any scientific activity encountered in the first post-war years, he forcefully restarted research on a broad front—a second thrust of activities that resulted in more than 200 publications over the next 20 years. He not only continued the topics that had been untowardly curtailed by the war, such as work on the Schardinger dextrins[38] and their inclusion compounds[39], on basic stereochemical problems that still had to be resolved[40]—a notable paper being on "the configuration of methyl ethyl propyl methane in relation to glucose"—and on flavonoid tannins[20], but, in a most intense way, he resumed research on lignin[41,42]. The structures of the primary building blocks (coniferyl alcohol **12**, for example) were unequivocally established, followed by the structural elucidation of a whole series of secondary fragments, such as **14**, **15**, and **16**, providing basic insights into the framework of this complex macromolecule. Yet only an entirely new approach to the lignin problem, *i.e.* the utilization of ^{14}C-labeled coniferin (**13**) in field experiments, and the isolation, characterization, and degradation of the then radioactively marked lignin gradually unfolded a lucid picture of its molecular architecture. This tenacious, and for the time truly pioneering, work cannot be given proper appreciation in these pages devoted to carbohydrate chemistry, but it bears witness to the unusual scope of its originator that, at the age of 80, he still had the vigour and productive capacity to conceive a constitutional scheme for the lignin of spruce that contained all the experimental data elaborated in more than four decades and provided not only the basic outline for its structure, but also the pathways for its biochemical formation in the plant[43].

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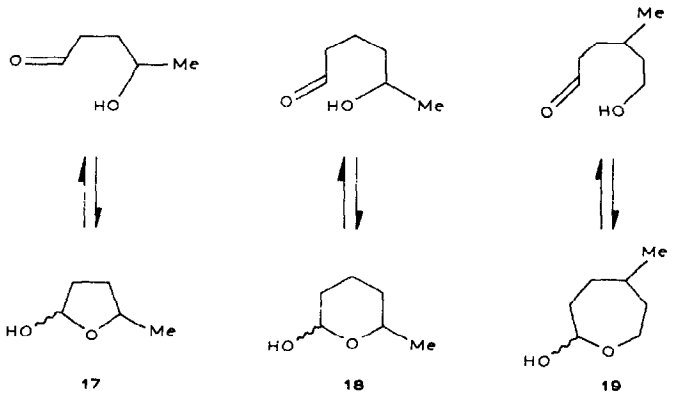
When he returned in 1919, after four years as an artillery officer in World War I, Helferich resumed his position as "Unterrichtsassistent" at the Chemical Institute in Berlin. In his first independent research he tackled[44–46] the basic question of the ring-chain tautomerism (then called "Cyclo-Oxo-Desmotropie") of hydroxyaldehydes **17–19**, with the clearly sugar-oriented aim of finding out "whether the properties attributed to the γ-hydroxyl in aldoses are also recognizable in simple γ-hydroxyaldehydes"[44]. On the basis of molecular-refraction data and the ready conversion of **17–19** into cyclic methyl acetals rather than their open-chain dimethyl derivatives — a question unequivocally resolvable by C and H analysis — the presence of the cyclic forms of the free aldehydes could be clearly delineated. This unmistakably pointed towards pyranoid (and septanoid) forms of the sugars at a time when the furanoid structure of Tollens (1883) was uniformly accepted and caused Helferich to state, in distinct realization of the consequences of his findings: "There is no justification anymore to assume the 1,4-ring for the cyclo-form of free sugars and their derivatives (in particular glucosides and disaccharides) without special proof; instead, the formation of a 1,5-ring must be taken into consideration much more strongly"[45]. Some years later, Haworth, Hirst,



17 18 19

and others proved the correctness of this conclusion.

Two other main lines of research which were to be pursued through all the future stations of Helferich's career — professorships in Frankfurt (1922), Greifswald (1925), Leipzig (1930), and Bonn (from 1945 on) — originated in Berlin: work directed towards the synthesis of di- and oligo-saccharides, and investigations on the glycosidases of sweet-almond emulsin. The latter started[47] in 1921, with the obtention of standardized enzyme preparations, *e.g.* "Helferich's Rohferment"[48], which, *via* further purification, could be brought to β-D-glucosidase values as high as 16. These preparations have been extensively used for determining the susceptibility to cleavage of glycosides in relation to the configuration of the sugar, its ring size, and its aglycon, the discovery of the pronounced difference in the hydrolysis rates of *o*- and *p*-substituted phenyl glycosides being a particularly noteworthy result[49]. An incipient cooperative effort with Pigman, who had joined Helferich's group at Leipzig in 1938, and Isbell — there are five joint publications[50] in 1939 — was rudely curtailed by the outbreak of World War II. Whereas these researches led to important early insights into the specificity of the glycosidases, unsuccessful attempts to separate the β-D-glucosidase from the β-D-galactosidase in almond emulsin also produced conflicting views on the identity of the two enzymes[51]. The controversy over the two views — a single enzyme cleaving β-D-glucosides as well as β-D-galactosides *versus* two enzymes of very similar properties — was resolved in favor of the latter possibility only in 1965. Then, Helferich and Kleinschmidt[52] succeeded, *via* the substantially improved purification techniques available, in crystallizing component B with a β-D-glucosidase value 46 times higher than that of the "Rohferment".

The basic problems involved in the synthesis of di- and oligo-saccharides—the latter a term proposed[53] by him in 1930—was Helferich's major preoccupation throughout his unusually long scientific life, his first publication[9] on this topic appearing in 1911, his last 63 years later[54]. Already in his dissertation, which was concerned with the preparation of some long-chain *n*-alkyl pyranosides[9], he had

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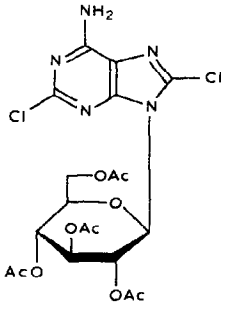
noted a curious double-melting behavior by some of the glycosides, inexplicable at the time, but later (1938) recognized as evidence for the formation of thermotropic liquid crystals[55]. In 1914, still with E. Fischer, Helferich pioneered the synthesis of purine nucleosides, when the silver salt of 2,8-dichloroadenine was reacted with acetobromoglucose to give **20** which, by appropriate reactions, could be converted into 9-β-D-glucopyranosyladenine (**21**)[12]. Many present day procedures[56] have their origin in this classical Fischer–Helferich method.

In the clear realization that the synthesis of di- and oligo-saccharides depended on the development of suitable monosaccharide derivatives having only



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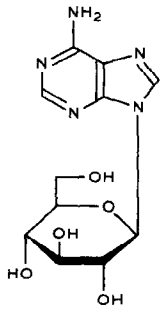

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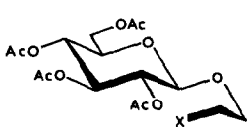
one free hydroxyl group, and on the improvement of glycosylation procedures, Helferich directed his efforts towards both of these basic issues, with remarkable results. The triphenylmethyl (trityl) group was introduced[57] already in 1923, and due to its high selectivity for primary hydroxyl groups and its ready removal without affecting acyl functions elsewhere in the molecule it was rapidly incorporated into the preparative arsenal not only of carbohydrate chemists—130 trityl sugars had accumulated when Helferich's comprehensive review[58] appeared in 1948—but of organic chemists in general, becoming the forerunner of a plethora of bulky protective groups. In turn, the availability of 1,2,3,4-blocked monosaccharides *via* this method led the way to the preparation of methyl gentiobioside[59], and, in 1926, of gentiobiose itself[60] *via* the reaction of acetobromoglucose with 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose. Thus gentiobiose became the first disaccharide of natural origin to be isolated in a pure state through a true chemical synthesis. A series of others were to follow, such as primeverose[61] and melibiose[62], along with a number of tri- and tetra-saccharides[62,63].

Another important outgrowth of this work was the recognition[13,64] that the formation of water during glycosylation effects hydrolysis of the educt, thereby making the addition of a desiccant imperative. This resulted in a considerably improved version[65] of the Koenigs–Knorr reaction, which has been termed "the Helferich modification"[66]. It comprises the condensation of acylglycosyl halides with alcohols in aprotic polar media (nitromethane or acetonitrile), with

mercury(II) cyanide as the catalyst and acceptor of hydrogen halide. The method usually provides high yields of glycosides and therefore has found wide application[66], despite its low stereoselectivity. Various other aspects of glycoside synthesis were also studied, *e.g.* the activation of the anomeric center by tosylation[67], and the preparation, *via* their respective haloethyl derivatives **22**, of *trans*- and *cis*-fused, 1,2-anellated, dioxane-type glycosides[68] (**23** and **24**). These showed an extreme acid stability that was to puzzle a good many later investigators after such structures were revealed in Nature. Another remarkable approach to systems of this type was uncovered in the reaction of tri-*O*-acetyl-D-glucal with phenanthrenequinone in boiling benzene under ultraviolet light, forming adduct **25** in up to 50% yield[69]. Since **25** can be ozonized readily to give diphenic acid and glucose, such adducts can serve as a means of hydroxyl-group protection at C-1 and C-2 of an aldose.

Many other lines of investigation were being pursued simultaneously in Helferich's laboratories. At Greifswald (1925) (ref. 70) he had commenced a study



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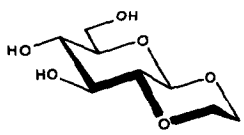

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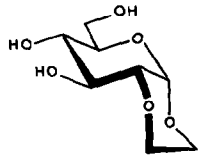

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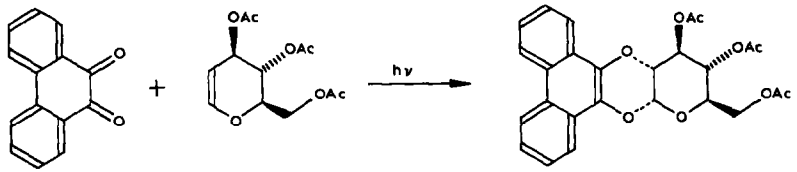

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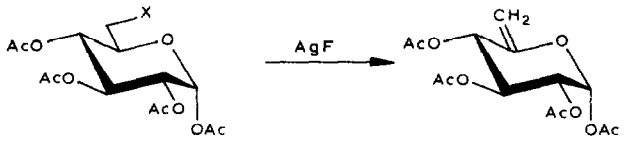
on terminally halogenated sugars, derivatives of special utility on account of their ready displacement reactions. This work also led — unintentionally for sure — to the first 5,6-unsaturated hexose derivative (**26**) on attempts to induce fluorination at C-6 with silver fluoride[71,72]. Similarly notable are the introduction, in 1937, of the methanesulfonate group[73] as an attractive leaving group that in many ways proved advantageous over the tosyloxy function, inaugurated by Freudenberg 15 years earlier. A good many other topics were pursued, extending into noncarbohydrate and even inorganic themes, the altogether 328 publications that emanated from Helferich's laboratory[4] giving ample evidence of the prolific flow of his ideas, and the power of his dynamic personality in realizing them.



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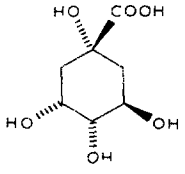
HERMANN O. L. FISCHER

Whilst Helferich was not much of a traveller on the scientific circuit—unlike Freudenberg he never visited the U.S., or Japan— the scientific career of Hermann Fischer was to follow an unusually cosmopolitan course. Returning at the end of 1918 from 4 years of service in a chemical warfare unit, he started redeveloping his career in chemistry, a task that was made all the more difficult by the devastating loss of his father in July 1919. However, he succeeded in gathering together a small research group, with which he gradually developed two main lines of research. One, in continuation of the earlier work with his father on orsellinic depsides[11], was the study of the structure and absolute configuration of quinic acid; the other dealt with the difficult chemistry of the trioses and related compounds.

The first publication[74] on quinic acid (1921) described a series of novel derivatives, but left the position of one of the hydroxyl groups uncertain. The available information together with other observations induced Karrer[75] to propose what appeared to be a satisfactory structural formula for the compound. After thorough reinvestigation, however, Fischer succeeded in disproving Karrer's proposal in favor of the correct structure[76] (**27**). This in turn led to the proper structure for chlorogenic acid (**28**) (ref. 77), a coffee bean- and cinchona bark-derived depside of caffeic acid and quinic acid. These studies were extended to shikimic acid during the period (1932–1937) in Basel, where Fischer had moved mainly on account of



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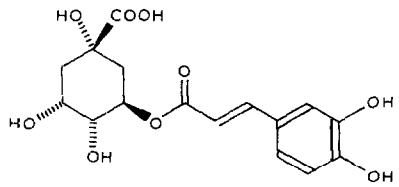

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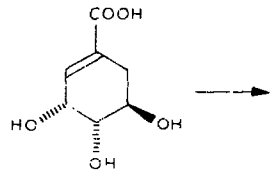

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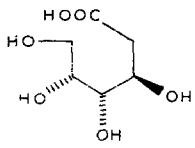

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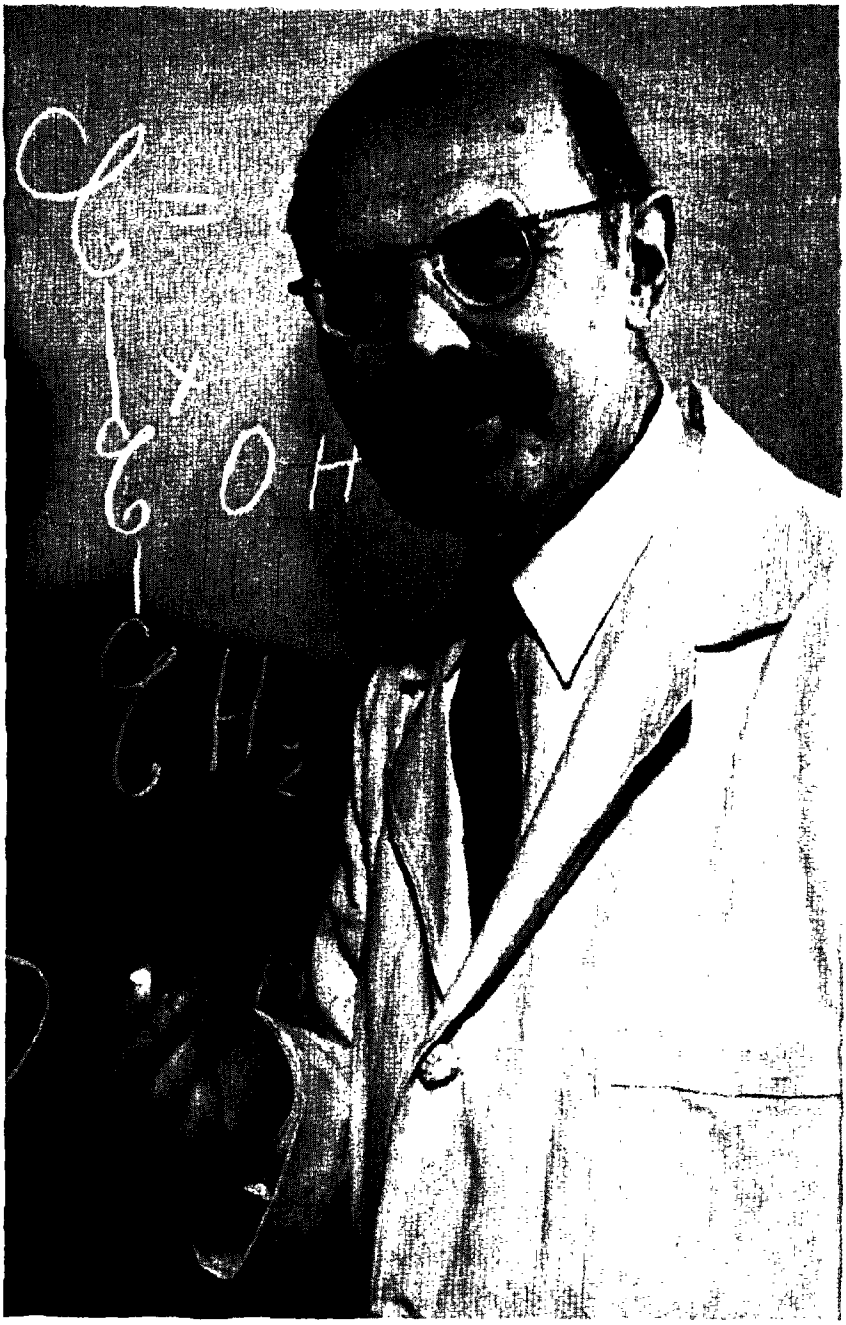
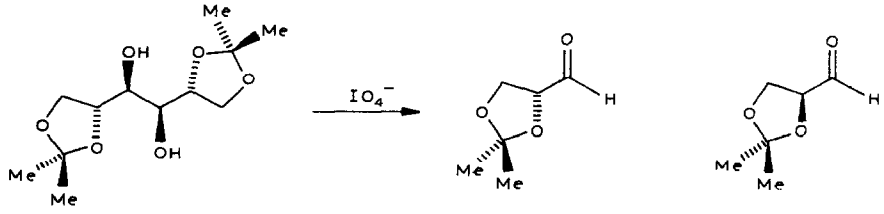


HERMANN O. L. FISCHER

1888–1960

his strong disapproval of the threatening political developments in Germany. By a brilliant series of experiments that converted shikimic acid into 2-deoxy-D-*arabino*-hexonic acid (**29**→**30**), its absolute configuration could be established[78]. The real reward for this meticulous work, however, was to come some 15 years later when a sample prepared by Fischer enabled B. D. Davis[79] to identify shikimic acid as a major biosynthetic intermediate in the formation of aromatic amino acids.

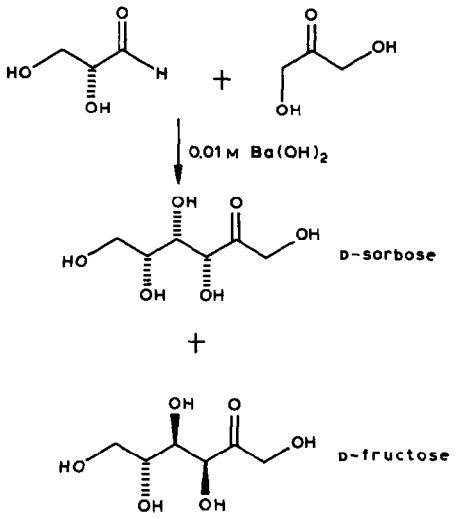
Parallel to the work on plant acids, a second major line of research had been initiated in Berlin: the exploration of the difficult chemistry of the trioses and related two-, three-, and four-carbon compounds. An important early achievement of this work, realized with the expert help of Erich Baer, was the preparation of DL-glyceraldehyde 3-phosphate in the form of its crystalline calcium salt[80]. With this compound it was soon shown in the laboratories of Otto Warburg, Gustav Embden, and Otto Meyerhof, that only the D enantiomer is fermentable, and, in fact, is the key three-carbon intermediate in fermentation and glycolysis as well. The fruitful collaboration with E. Baer continued in Switzerland, and in Canada, where Fischer had accepted a research professorship at the Banting Institute of the University of Toronto in 1937, and produced many more results of great significance. One, for example, was the oxidative cleavage of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**31**) into 2,3-*O*-isopropylidene-D-glyceraldehyde (**32**) (ref. 21), which today has developed into one of the most popular, enantiomerically pure three-carbon synthons[82]. Inasmuch as free D-glyceraldehyde could be readily obtained from **32**, the totally fermentable D-glyceraldehyde 3-phosphate and its enantiomer (*via* L-mannitol[83] and **33**) were made available for biochemical studies.



31 32 33

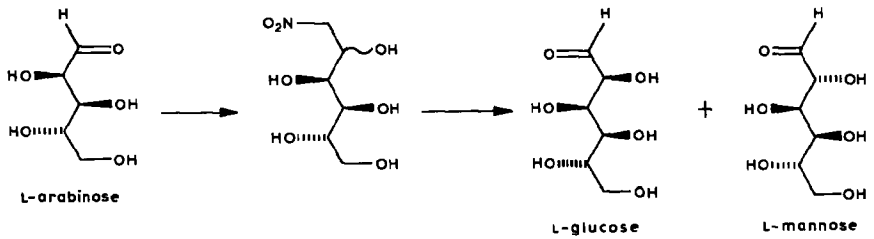
Similarly, in a remarkably stereoselective aldol addition that today would be labeled "biomimetic", dihydroxyacetone and D-glyceraldehyde were shown under very mild basic conditions to give D-fructose and D-sorbose in high yields, with practically complete exclusion of the D-*psico* and D-*tagato* isomers[84].

The two enantiomeric *O*-isopropylidene glyceraldehydes **32** and **33** also provided, through reduction, the 2,3-*O*-isopropylidene-D- and L-glycerols, which opened up the entire field of asymmetric mono- and di-glyceride synthesis, as well as making accessible synthetic ethers and phosphates of glycerol in their enantiomerically pure forms[85]. Extensive work on other phosphorylated lower-carbon sugars was to follow at the University of California, Berkeley (from 1948 on), where new, practical methods were devised for the preparation of phosphoenolpyruvic acid[86],



the 2- and 3-phospho-D-glyceric acids[87], and the 4-phosphates of D-erythritol and of D-erythrose[88]. The biochemical significance of the latter was soon to be established by Sprinson, who demonstrated, in *E. coli*, its condensation with phosphoenolpyruvate to give 5-dehydroshikimic acid[89].

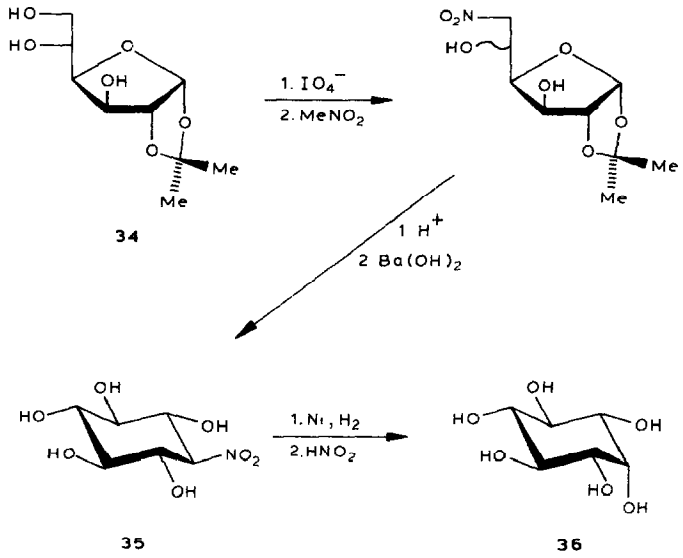
Other significant contributions from Fischer's laboratory were studies on the addition of nitromethane to *aldehydo* sugars, yielding 2-epimeric nitroalditols that



could be reconverted to sugars *via* the Nef reaction[90]. Thus, a new and general method of ascending the sugar series was made available which in many instances proved to be more convenient than the classical cyanohydrin method—the preparation of L-glucose and L-mannose from L-arabinose being a notable example[91]. In an intriguing adaptation of this reaction to the pentodialdose formed on periodate oxidation of 1,2-*O*-isopropylidene-D-glucose (**34**), nitromethane was first used for C-extension, and, subsequently, for an intramolecular addition to yield the nitroinositols having the *scyllo* (**35**), *myo*-1, and *muco*-3 configurations[92]. These are convertible by reduction to the corresponding inosamines, of which the *scyllo* isomer could be deaminated, albeit in modest yield[93], to *myo*-inositol (**36**), thus formally

completing the synthesis of this cyclitol from D-glucose.

Undoubtedly, this cyclizing nitromethane addition was the conceptual forerunner of later work with the dialdehydes arising from the periodate oxidation of the common glycosides[94], which developed the "cyclization of dialdehydes with nitromethane" as a most efficient procedure for the preparation of 3-nitro- and hence 3-amino-3-deoxy derivatives of pentoses, hexoses, and inositols[95]. This field still has its attractions today as evidenced by several contributions in this



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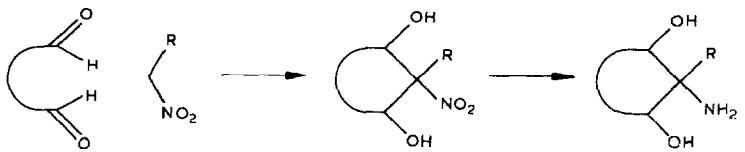
commemorative issue describing novel compounds of biochemical interest, in resumption and/or continuation of Fischer's own modestly stated evaluation: "All my life I have essentially remained an organic chemist with a strong predilection for synthesizing compounds useful for biochemical purposes"[5].

EPILOGUE

In view of the prodigious series of significant and highly original contributions that have left their mark not only on carbohydrate chemistry over the past 60 years but in a most profound way on organic chemistry and biochemistry as well, it is a



R = H, Me, Et, COOR

tribute to the intelligence, imagination, and assiduousness of these three distinguished scientists that they could achieve all of this despite the adverse circumstances they had to cope with during their lifetimes: a delayed start to their academic careers, the emotional shocks of two wars—Fischer lost his two brothers in the First World War, Helferich his only son, Freudenberg his two sons in the Second, the disturbed economic and political conditions following each of these wars, the frequent moves with attendant adjustments, and the handicap — or was it a challenge? — of inevitably being expected to live up to the reputation of their illustrious teacher.

Their individual achievements were widely recognized during their lifetimes and many honours were bestowed upon them. To mention a few, the Gesellschaft Deutscher Chemiker awarded the "Emil-Fischer-Medal" to Helferich (1951) and Freudenberg (1952), and the "Adolf-von-Baeyer-Denkmünze" to Fischer in 1955. On their 70th birthdays they were honoured by special publications, *i.e.* a "Festschrift" in *Angewandte Chemie* for Freudenberg[96], and Helferich[97], and in the case of H. O. L. Fischer an anniversary issue of the *Archives of Biochemistry and Biophysics*[98], to which many of the world's foremost chemists and biochemists contributed. This was thirty years ago. Yet even today, a hundred years after their births, the spirit and imagination of the three chemists, the concepts they evolved, their relentless endeavour in pursuing self-imposed scientific aims and, not the least, the radiance of their imposing personalities continue to exert a lasting influence not only on those who had the privilege of knowing and being associated with them in one way or another, but on today's generation of new scholars as well through the marks they left on their science.

The papers published in this volume are a result of this still prevailing influence. Through their contributions the authors — some former students or academic associates, others, carbohydrate chemists whose work has been heavily influenced by the achievements of the three honorees — sought to express their respect and admiration for the importance of their achievements in the advancement of carbohydrate chemistry in this century.

REFERENCES

1 B. HELFERICH, "Karl Freudenberg zum 70. Geburtstag", *Angew. Chem.*, 68 (1956) 81–84.
2 F. CRAMER, "Leben und Werk von Karl Freudenberg", *Heidelberger Jahrbücher*, Vol. XXVIII, Springer-Verlag, Berlin/Heidelberg/New York, 1984, pp. 57–72.
3 H. BREDERECK, "Burckhardt Helferich zum 70. Geburtstag", *Angew. Chem.*, 69 (1957) 405–412.
4 H. STETTER, "Obituary B. Helferich", *Chem. Ber.*, 118 (1985) I–XIX.
5 H. O. L. FISCHER, "Fifty Years in the Service of Biochemistry", *Ann. Rev. Biochem.*, 29 (1960) 1–14.
6 J. C. SOWDEN, "Hermann Otto Laurenz Fischer", *Adv. Carbohydr. Chem.*, 17 (1962) 1–14.
7 W. M. STANLEY AND W. Z. HASSID, *Biographical Memoirs Natl. Acad. Sci. U.S.A.*, Vol. XL, Columbia University Press, New York, 1969, pp. 91–112.
8 E. FISCHER AND K. FREUDENBERG, *Justus Liebigs Ann. Chem.*, 372 (1910) 32–68.
9 E. FISCHER AND B. HELFERICH, *Justus Liebigs Ann. Chem.*, 383 (1911) 68–91.
10 E. FISCHER AND K. FREUDENBERG, *Justus Liebigs Ann. Chem.*, 384 (1911) 225–244; *Ber. Dtsch. Chem. Ges.*, 45 (1912) 915–935, 2709–2726; 46 (1913) 1116–1138, 2485–2504.

11 E. FISCHER AND H. O. L. FISCHER, *Ber. Dtsch. Chem. Ges.*, 46 (1913) 1138–1148; 47 (1914) 505–512; 768–780.
12 E. FISCHER AND B. HELFERICH, *Ber. Dtsch. Chem. Ges.*, 47 (1914) 210–235.
13 B. HELFERICH, *Angew. Chem.*, 63 (1951) 479.
14 K. FREUDENBERG, "Emil Fischer and His Contribution to Carbohydrate Chemistry", *Adv. Carbohydr. Chem.*, 21 (1966) 1–38.
15 K. FREUDENBERG, *Ber. Dtsch. Chem. Ges.*, 47 (1914) 2027–2037.
16 K. FREUDENBERG, *Ber. Dtsch. Chem. Ges.*, 52 (1919) 177–185; K. FREUDENBERG AND D. PETERS, *ibid.*, 53 (1920) 953–961; K. FREUDENBERG AND F. BLÜMMEL, *Justus Liebigs Ann. Chem.*, 440 (1924) 45–49.
17 O. TH. SCHMIDT AND W. MAYER, *Angew. Chem.*, 68 (1956) 103–115; F. SHAFIZADEH, *Adv. Carbohydr. Chem.*, 11 (1957) 267–271.
18 K. FREUDENBERG, *Ber. Dtsch. Chem. Ges.*, 52 (1919) 1238–1246; K. FREUDENBERG AND B. FICK, *ibid.*, 53 (1920) 1728–1736; K. FREUDENBERG AND T. FRANK, *Justus Liebigs Ann. Chem.*, 452 (1927) 303–315.
19 K. FREUDENBERG, *Tannin, Cellulose, Lignin*, Springer Verlag, Berlin, 1933.
20 K. FREUDENBERG AND K. WEINGES, *Justus Liebigs Ann. Chem.*, 590 (1954) 140–154; in T. A. GEISSMAN (Ed.), *The Chemistry of Catechins and Flavonoid Tannins*, Pergamon, 1962, pp. 197–216.
21 K. FREUDENBERG AND F. BRAUNS, *Ber. Dtsch. Chem. Ges.*, 55 (1922) 1339–1352; 56 (1923) 193–200; K. FREUDENBERG AND L. MARKERT, *ibid.*, 58 (1925) 1753–1760; 60 (1927) 2447–2458.
22 K. FREUDENBERG AND O. HUBER, *Ber. Dtsch. Chem. Ges.*, 58 (1925) 148–150.
23 K. FREUDENBERG, W. KUHN, AND I. BUMANN, *Ber. Dtsch. Chem. Ges.*, 63 (1930) 2381–2390; K. FREUDENBERG AND W. KUHN, *ibid.*, 64 (1931) 703–734.
24 K. FREUDENBERG, *Stereochemie*, F. Deuticke Verlag, Leipzig, 1933.
25 K. FREUDENBERG AND O. IVERS, *Ber. Dtsch. Chem. Ges.*, 55 (1922) 929–941; K. FREUDENBERG AND F. BRAUNS, *ibid.*, 55 (1922) 3233–3238; K. FREUDENBERG AND A. DOSER, *ibid.*, 56 (1923) 1243–1247.
26 K. FREUDENBERG AND K. RASCHIG, *Ber. Dtsch. Chem. Ges.*, 60 (1927) 1633–1636; 62 (1929) 373–383; *Adv. Carbohydr. Chem.*, 8 (1953) 107–215.
27 R. S. TIPSON, *Adv. Carbohydr. Chem.*, 8 (1953) 107–215.
28 K. FREUDENBERG, A. WOLF, E. KNOPF, AND S. H. ZAHEER, *Ber. Dtsch. Chem. Ges.*, 61 (1928) 1743–1750; 1751–1760.
29 K. FREUDENBERG AND E. BRAUN, *Naturwissenschaften*, 18 (1930) 393–395; K. FREUDENBERG AND H. SCHOLZ, *Ber. Dtsch. Chem. Ges.*, 63 (1930) 1969–1972.
30 K. FREUDENBERG AND H. EICHEL, *Justus Liebigs Ann. Chem.*, 510 (1934) 240–248; 518 (1935) 97–102; K. FREUDENBERG, *Umschau*, 46 (1942) 373–374.
31 K. FREUDENBERG, *Chem.-Ztg.*, 83 (1921) 666; *Ber. Dtsch. Chem. Ges.*, 54 (1921) 767–772.
32 K. FREUDENBERG, *Justus Liebigs Ann. Chem.*, 461 (May 1928) 130–131.
33 W. N. HAWORTH, *Helv. Chim. Acta*, 11 (June 1928) 534–548.
34 K. FREUDENBERG, "Von Emil Fischer zur molekularen Konstitution der Cellulose und Stärke", *Chem. Ber.*, 100 (1967) CLXXII–CLXXXVIII.
35 K. FREUDENBERG, G. BLOMQVIST, L. EWALD, AND K. SOFF, *Ber. Dtsch. Chem. Ges.*, 69 (1936) 1258–1266.
36 K. FREUDENBERG AND G. BOPPEL, *Ber. Dtsch. Chem. Ges.*, 71 (1938) 2505–2511; 73 (1940) 609–620.
37 K. FREUDENBERG, E. SCHAAF, G. DUMPERT, AND T. PLOETZ, *Naturwissenschaften*, 27 (1939) 850–853; K. FREUDENBERG AND H. BOPPEL, *Ber. Dtsch. Chem. Ges.*, 73 (1940) 609–620.
38 K. FREUDENBERG, E. PLANKENHORN, AND H. KNAUBER, *Justus Liebigs Ann. Chem.*, 558 (1947) 1–10; K. FREUDENBERG AND F. CRAMER, *Chem. Ber.*, 83 (1950) 296–304.
39 F. CRAMER, *Inclusion Compounds*, Springer Verlag, Heidelberg, 1954; *Angew. Chem.*, 68 (1956) 115–120.
40 K. FREUDENBERG, *Angew. Chem.*, 65 (1953) 259–260; K. FREUDENBERG AND W. LWOWSKI, *Justus Liebigs Ann. Chem.*, 587 (1954) 213–225; 594 (1955) 76–88, 141–147.
41 K. FREUDENBERG, "The Beginnings of My Work on Lignin", *Wood Sci. Technol.*, 1 (1967) 166–167.
42 K. FREUDENBERG, *Fortschr. Chem. Org. Naturst.*, 11 (1954) 43–82; *Angew. Chem.*, 68 (1956) 81–92; *Chem. Ber.*, 92 (1959) LXXXIX–XCVIII; *Nature*, 183 (1959) 1152–1159; *Pure Appl. Chem.*, 5 (1962) 9–20.

43 K. FREUDENBERG, *Holzforschung*, 18 (1964) 3–9; *Advances Chemistry Series*, v. 59, American Chemical Society, Washington, 1966, pp. 1–21.
44 B. HELFERICH, *Ber. Dtsch. Chem. Ges.*, 52 (1919) 1123–1131, 1800–1812.
45 B. HELFERICH AND T. MALKOMES, *Ber. Dtsch. Chem. Ges.*, 55 (1922) 702–708.
46 B. HELFERICH AND H. KÖSTER, *Ber. Dtsch. Chem. Ges.*, 56 (1923) 2088–2094; B. HELFERICH AND W. SCHÄFER, *ibid.*, 57 (1924) 1911–1917; B. HELFERICH AND F. A. FRIES, *ibid.*, 58 (1925) 1246–1251.
47 B. HELFERICH, *Hoppe-Seyler's Z. Physiol. Chem.*, 117 (1921) 159–171.
48 B. HELFERICH, S. WINKLER, R. GOOTZ, O. PETERS, AND E. GÜNTHER, *Hoppe-Seyler's Z. Physiol. Chem.*, 208 (1932) 91–100.
49 B. HELFERICH, *Ergebn. Enzymforsch.*, 7 (1938) 83–104; in J. B. SUMNER AND K. MYRBÄCK (Eds.), *The Enzymes*, Vol. I, Academic Press, New York, 1950, pp. 79–114.
50 B. HELFERICH, R. HILTMANN, AND W. W. PIGMAN, *Hoppe-Seyler's Z. Physiol. Chem.*, 259 (1939) 150–156; B. HELFERICH AND W. W. PIGMAN, *Ber. Dtsch. Chem. Ges.*, 72 (1939) 212–215; *Hoppe-Seyler's Z. Physiol. Chem.*, 259 (1939) 253–262; B. HELFERICH, W. W. PIGMAN, AND H. S. ISBELL, *ibid.*, 261 (1939) 55–60; 189–192.
51 K. NISIZAWA AND Y. HASHIMOTO, in W. W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, Vol. IIA, Academic Press, New York, 1970, pp. 258 ff.
52 B. HELFERICH AND T. KLEINSCHMIDT, *Hoppe-Seyler's Z. Physiol. Chem.*, 340 (1965) 31–45; 348 (1967) 753–758; 349 (1968) 25–28.
53 B. HELFERICH, E. BÖHM, AND S. WINKLER, *Ber. Dtsch. Chem. Ges.*, 63 (1930) 989–998.
54 B. HELFERICH, W. M. MÜLLER, AND S. KARBACH, *Justus Liebigs Ann. Chem.*, (1974) 1514–1521.
55 C. R. NOLLER AND W. C. ROCKWELL, *J. Am. Chem. Soc.*, 60 (1938) 2076–2077.
56 J. A. MONTGOMERY AND H. J. THOMAS, *Adv. Carbohydr. Chem.*, 17 (1962) 313–341.
57 B. HELFERICH, P. E. SPEIDEL, AND W. TOELDTE, *Ber. Dtsch. Chem. Ges.*, 56 (1923) 766–770.
58 B. HELFERICH, *Angew. Chem.*, 41 (1928) 871–875; *Adv. Carbohydr. Chem.*, 3 (1948) 79–111.
59 B. HELFERICH AND J. BECKER, *Justus Liebigs Ann. Chem.*, 440 (1924) 1–18; B. HELFERICH AND W. KLEIN, *ibid.*, 450 (1926) 219–229.
60 B. HELFERICH, K. BÄUERLEIN, AND F. WIEGAND, *Justus Liebigs Ann. Chem.*, 447 (1926) 27–37.
61 B. HELFERICH AND H. RAUCH, *Justus Liebigs Ann. Chem.*, 455 (1927) 168–172.
62 B. HELFERICH AND H. BREDERECK, *Justus Liebigs Ann. Chem.*, 465 (1928) 166–184.
63 B. HELFERICH AND W. SCHÄFER, *Justus Liebigs Ann. Chem.*, 450 (1926) 229–237.
64 B. HELFERICH AND R. GOOTZ, *Ber. Dtsch. Chem. Ges.*, 64 (1931) 109–114.
65 B. HELFERICH AND B. WEISS, *Chem. Ber.*, 89 (1956) 314–321; B. HELFERICH AND J. ZIRNER, *ibid.*, 95 (1962) 2604–2611.
66 A. F. BOCHKOV AND G. E. ZAIKOV, *Chemistry of the O-Glycosidic Bond*, Pergamon, Oxford/New York, 1979, pp. 7 ff. and 20 ff.
67 B. HELFERICH AND R. GOOTZ, *Ber. Dtsch. Chem. Ges.*, 62 (1929) 2788–2792.
68 B. HELFERICH AND J. WERNER, *Ber. Dtsch. Chem. Ges.*, 75 (1942) 949–951, 1446–1452; 76 (1943) 595–599; B. HELFERICH, *ibid.*, 80 (1947) 158–162.
69 B. HELFERICH AND E. VON GROSS, *Chem. Ber.*, 85 (1952) 531–535; B. HELFERICH, E. MULCAHY, AND H. ZIEGLER, *ibid.*, 87 (1954) 233–237; B. HELFERICH AND M. GINDY, *ibid.*, 87 (1954) 1488–1491.
70 B. HELFERICH, G. SPROCK, AND E. BESLER, *Ber. Dtsch. Chem. Ges.*, 58 (1925) 886–891.
71 B. HELFERICH AND R. HILTMANN, *Justus Liebigs Ann. Chem.*, 531 (1937) 160–175.
72 B. HELFERICH AND E. HIMMEN, *Ber. Dtsch. Chem. Ges.*, 61 (1928) 1825–1835; 62 (1929) 2136–2141.
73 B. HELFERICH AND A. GNÜCHTEL, *Ber. Dtsch. Chem. Ges.*, 71 (1938) 712–720; B. HELFERICH, H. DRESSLER, AND R. GRIEBEL, *J. Prakt. Chem.*, 153 (1939) 285–299.
74 H. O. L. FISCHER, *Ber. Dtsch. Chem. Ges.*, 54 (1921) 775–784.
75 P. KARRER, R. WIDMER, AND P. RISO, *Helv. Chim. Acta*, 8 (1925) 195–202.
76 H. O. L. FISCHER AND G. DANGSCHAT, *Ber. Dtsch. Chem. Ges.*, 65 (1932) 1009–1031.
77 H. O. L. FISCHER AND G. DANGSCHAT, *Ber. Dtsch. Chem. Ges.*, 65 (1932) 1037–1040.
78 H. O. L. FISCHER AND G. DANGSCHAT, *Helv. Chim. Acta*, 17 (1934) 1200–1207; 18 (1935) 1204–1206, 1206–1213; 20 (1937) 705–716; *Naturwissenschaften*, 26 (1938) 562–563.
79 B. D. DAVIS, *Adv. Enzymol.*, 16 (1955) 287–295.
80 H. O. L. FISCHER AND E. BAER, *Ber. Dtsch. Chem. Ges.*, 65 (1932) 337–345.
81 H. O. L. FISCHER AND E. BAER, *Helv. Chim. Acta*, 17 (1934) 622–632; *J. Biol. Chem.*, 128 (1939) 463–473.

82 J. JURCZAK, S. PIKUL, AND T. BAUER, *Tetrahedron*, 42 (1986) 447–488.
83 E. BAER AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 61 (1939) 761–765.
84 H. O. L. FISCHER AND E. BAER, *Helv. Chim. Acta*, 19 (1936) 519–532.
85 H. O. L. FISCHER AND E. BAER, *Chem. Rev.*, 29 (1941) 287–316; *J. Am. Chem. Soc.*, 67 (1945) 2031–2037; *J. Biol. Chem.*, 170 (1947) 337–342.
86 E. BAER AND H. O. L. FISCHER, *J. Biol. Chem.*, 180 (1949) 145–154.
87 C. E. BALLOU AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 76 (1954) 3188–3193; 77 (1955) 3329–3331; 78 (1956) 1659–1661.
88 D. L. MACDONALD, H. O. L. FISCHER, AND C. E. BALLOU, *J. Am. Chem. Soc.*, 77 (1955) 5967–5970; 78 (1956) 3720–3722.
89 P. R. SRINAVASAN, M. KATAGIRI, AND D. B. SPRINSON, *J. Am. Chem. Soc.*, 77 (1955) 4943–4944.
90 J. C. SOWDEN AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 66 (1944) 1312–1314; 67 (1945) 1713–1715; 68 (1946) 1511–1513; J. C. SOWDEN, *Adv. Carbohydr. Chem.*, 6 (1951) 291–318.
91 J. C. SOWDEN AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 69 (1947) 1963–1965; J. C. SOWDEN, *Methods Carbohydr. Chem.*, 1 (1962) 132–136.
92 J. M. GROSHEINTZ AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 70 (1948) 1476–1479, 1479–1484; F. W. LICHTENTHALER, *Chem. Ber.*, 94 (1961) 3071–3085.
93 T. POSTERNAK, *Helv. Chim. Acta*, 33 (1950) 1597–1605.
94 H. H. BAER AND H. O. L. FISCHER, *Proc. Natl. Acad. Sci. U.S.A.*, 44 (1958) 991–996; *J. Am. Chem. Soc.*, 81 (1959) 5184–5189; A. C. RICHARDSON AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 83 (1961) 1132–1139; F. W. LICHTENTHALER AND H. O. L. FISCHER, *ibid.*, 83 (1961) 2005–2012.
95 F. W. LICHTENTHALER, *Angew. Chem. Int. Ed. Engl.*, 3 (1964) 211–264; H. H. BAER, *Adv., Carbohydr. Chem. Biochem.*, 24 (1969) 78–97.
96 *Angew. Chem.*, 68, No. 3 (1956, pp. 81–132).
97 *Angew. Chem.*, 69, No. 12 (1957, pp. 405–444).
98 *Arch. Biochem. Biophys.*, 78, No. 2 (1958, pp. i–iv, 275–602).