Adventures with Carbons 11, 12, 13 and 14

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Received: 4 July 2011 / Accepted: 24 August 2011 / Published online: 13 September 2011
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Abstract I provide here a glimpse of my involvement with different isotopes of carbon. In my 65 years of synthetic work with C-12, I had experience working with C-11 (one year, 1942–1943), C-13 (one year, 1999) and C-14 (67 years, 1943–2009). I have also included a postscript dealing with my 1951 communication on the 5-carbon intermediate in photosynthesis.

Keywords Roland Douce · Martin Kamen · Gilbert N. Lewis · Linus Pauling · Sam Ruben · Glenn T. Seaborg · The Rat House

Background

My research between 1937 and 1997 involved four isotopes of carbon. My undergraduate research in organic chemistry under Professor T.D. Stewart at the University of California at Berkeley involved the synthesis and study of trinitrotriphenylmethane. One purpose was to provide Professor Gilbert N. Lewis and his postdoctoral collaborator, Glenn T. Seaborg, this compound for their study on the color of molecules. The acidity of its central hydrogen interested Lewis, much in the same way as it did my teacher, Linus Pauling at Caltech, Pasadena (see Kalm 1994). In basic solution, a gorgeous blue salt forms and is soon oxidized on exposure to oxygen.

My own research involved a study on the kinetics of oxidation of the trinitrotriphenylmethane salt in acetonitrile solution. I preserved some of this blue solution by sealing a flask of it; it has been stable for over 60 years! Further organic synthetic research from 1939 to 1942 at Caltech provided more experience with the reactions of organic molecules of carbon-12. After presentation of my thesis work, Linus Pauling asked me to write the equation for the kinetics of decay of a radioactive isotope on the black board—a subject that had no relation to my thesis or to studies at Caltech. I managed to write the generalized differential equation but Pauling said nothing about his reason for asking the question. (See Pauling (1940) for his ideas on *The Chemical Bond*.) Two weeks later I received a letter from Professor Joel Hildebrand offering me a position as Instructor in the Chemistry Department at the

Editor's Note: This letter was written at my invitation. I refer the readers to several recent items that have dealt with Andrew A. Benson and his contributions (see the reference list): (1) Buchanan et al. (2007) edited a special issue of Photosynthesis Research honoring Andy Benson; (2) Lichtenthaler et al. (2008) wrote a short article on a dinner in Paris, that celebrated Benson's 90th birthday; (3) Morton (2008; see pp. 3–47) discussed, in general terms, the story of the path of carbon in photosynthesis; (4) Govindjee (2010) wrote an article honoring Benson on his 93rd birthday. (5) A DVD titled "Dr. A.A. Benson 2010" was produced by S. Miyachi (<http://www.marinebio-miyachi.com> [the web site is in Japanese]; e-mail: info@marinebio-miyachi.com). (6) Nickelsen (2010; see pp. 250–317) discussed, in scientific and historical terms, the story of the path of carbon in photosynthesis. (7) British Broadcasting Corporation (BBC) produced a video (Botany: A Blooming History; Episode 2: The Power of Plants <http://www.bbc.co.uk/programmes/p00hhnnf> that included Benson's contributions. (8) ISPR (International Society of Photosynthesis Research) <http://www.photosynthesisresearch.org/Default.aspx?pageId=216517> gives the "Melvin Calvin-Andrew Benson" Award every 3 years at their International Congress. The final copy of this *Historical Corner Letter* was read, edited, and accepted by Bob Buchanan—Govindjee, founding Historical Corner Editor of Photosynthesis Research.

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University of California Berkeley, with a salary of \$2,000 per year. Apparently, Pauling and Wendell Latimer, Dean of the College of Chemistry and Chemical Engineering at UC Berkeley had arranged this appointment. As an Instructor, I taught courses in synthetic organic chemistry. Clearly, Pauling and Latimer had already planned that I should work with Sam Ruben and Martin Kamen in their research on the path of carbon in photosynthesis (see Gest 2005a for Kamen; and Gest 2005b for Ruben).

Sam and Martin were excellent physical chemists who found themselves in the middle of an adventure in plant biochemistry, the mechanism of carbon fixation and reduction in photosynthesis. Clearly, they needed organic chemistry expertise in their quest. At this time they were not involved in the classified research involving “atomic energy/power.” I had been made aware of nuclear fission since the morning of January 13, in 1939, when Luis Alvarez came into his 11 am physics lecture in a state of shock, engendered by the news of Hahn and Meitner’s report of their discovery of nuclear fission. This discovery had to be verified at once. That momentous morning, his lecture on optics was really an excited report of the discovery in Germany that changed the course of history.

During my graduate work in the following 3 years at Caltech, the discovery had resulted in an unpublicized national atomic energy program, completely classified; this was later known as the Manhattan Project (see Kelly 2007). In the Berkeley chemistry department it was known as the Metals Project and occupied the closed third floor of Gilman Hall where Glenn Seaborg had a small laboratory. No one discussed what was going on there. Sam Ruben once mentioned atomic energy to me but that was as far as it went. As I arrived in Latimer’s office, June 1942, he directed me to a little laboratory in the Rat House and to Sam Ruben.

The C-11 work: Ruben and Kamen

Sam Ruben knew that I had no experience with photosynthesis. He handed me his copy of Burris, Stauffer and Umbreit’s ‘Manometric Methods’ (see Umbreit et al. 1957) and showed me the Warburg apparatus on the third floor of the Rat House (Kalm 1994) where he grew the green alga, *Chlorella*. Soon the experiments began.

This building was called ‘The Rat House’ in light of its previous use by biologists for the culture and experiments with rats; it was built of wood in 1915 with three floors; we entered it from the West doorway midway between the street-level floor and the second floor.

The experiments always began at about 8:00 pm, since Martin Kamen needed the time for bombardment of his boron target after the physicists on the “37 inch” cyclotron

had left for supper. When the bombardment was completed, a target was removed and connected to an evacuated “Aspirator” (Fig. 1), which removed gaseous $C^{11}O_2$ and $C^{11}O$ from the target. The Aspirator was coupled to a copper oxide-filled quartz tube within a fired furnace for conversion of the gas mixture to pure $C^{11}O_2$ for the photosynthesis experiments. At that point, the dash began from the cyclotron to the Rat House and Sam’s waiting arms followed the demand that the ‘radioactive Martin,’ “leave at once.”

At first I was a helper while the more experienced Peter Yankwich, Charlie Rice and Mary Belle Allen performed their preplanned duties. Ruben managed the stopcocks and transfers from the liquid air-cooled spiral trap for the $C^{11}O_2$ to the waiting algae.

In a wartime research project Sam became involved in meteorology of toxic gas clouds. Working closely with him, I prepared steel containers with valves and filled them with liquid phosgene (b.p. $8^\circ C$) provided in 150 ml sealed ampoules for him. (Note: The Rat House had no fume hoods, only large double hung windows.) Later, I managed my synthesis of C11-phosgene for animal experiments to determine the protein product and the mechanism that rendered phosgene so toxic.

Having produced C^{11} -phosgene in 20 min, Sam and I (Ruben and Benson 1943) performed an experiment with a small rat, intending to demonstrate the presence of the phosgene’s C-11 in the animal’s lung fluid protein. I intended to show that the phosgene’s double acid chloride [Cl–CO–Cl] structure could bind two proteins together or link two amino groups in a single protein to alter its conformation and hence develop its antigenicity. Sadly, my conscription to Civilian Public Service (CPS) by my Pasadena Draft Board, and Sam’s untimely death by phosgene inhalation terminated this effort (see Benson 2005).

Fig. 1 Author (AAB) holding the ‘aspirator’ that was used by Martin Kamen. Source: Fig. 8 in Govindjee (2010)

The C-14 work

In my studies of C-14 (see Jolly 1987), carbon fixation and reduction designed to follow the path of carbon in photosynthesis, many C-14 syntheses and identification experiments were performed and reported in a long series of publications (see overviews in Bassham 2005; Benson 2002, 2005, 2010). The first such Report was written in 1943 at Galena Creek on the Sonora Pass highway in Nevada. Unfortunately, it was not submitted to the Journal of the American Chemical Society as planned. It described results of my experiments in the Rat House of the first use of C-14 in following the path of carbon in photosynthesis by using immiscible solvent partition measurements in recognizing properties of the products necessary for their identification.

The C-13 work

In 1997, I synthesized C-13 glycolic acid from C-13 formaldehyde and sodium cyanide in tetrahydrofuran. With Roland Douce and his skilled collaborators, it was administered to live cultured sycamore cells in the field of the 400 MHz NMR spectrometer in the Center for Atomic Energy, Grenoble, France, and the spectrum of the products evaluated. At the same time, the metabolism of C-13 methanol (Gout et al. 2000) revealed the production of C-13 methyl glucoside. This was later found to stimulate plant growth (Nonomura and Benson 1992).

Postscript

As a postscript, I would like to mention a paper of mine (Benson 1951) that was the first paper dealing with the identification of a 5-C sugar, ribulose. Appendix 1 reproduces an e-mail that I wrote to Govindjee; it may be of importance to historians of photosynthesis.

Acknowledgments I appreciate the valuable editorial suggestions and corrections by John F. Kern, of Winnetka, IL. I am grateful to Bob Buchanan, Dee Benson and Carole Mayo for their support. I thank Govindjee for his invitation, his extensive editing (especially in providing the reference list), his patience and above all his ever-lasting persistence and encouragement that has led to the completion of this letter.

Appendix 1

(Source: E-mail of A.A. Benson to Govindjee, December 5, 2010; see Benson 1951)

“*Nature’s Plant Assembly Line*. Ribulose biphosphate is the compound that reacts with CO₂ and produces 2

molecules of the first product of CO₂ fixation. For several years [up to 1951], we had searched for a 2-carbon compound that could add CO₂ to yield the first product of photosynthesis, glyceric acid 3-phosphate. *The search was futile*. By comparing the composition of the illuminated algae without CO₂ and those with ample CO₂, we observed a minimal concentration of a phosphate ester when ample CO₂ was present, and a maximal concentration of that compound when CO₂ was not available. This indicated that the compound might be reacting with CO₂. Hence, chemical identification of the compound became essential. Enzymatic hydrolysis of that compound yielded a sugar, one carbon smaller than glucose or fructose. There were several possibilities including ribose, arabinose, and ribulose.

The paper chromatographic position of the C-14-labeled sugar corresponded precisely with that of ribulose, prepared by epimerization of ribose or arabinose in pyridine. The radioactive sugar resisted bromine oxidation, but was cleaved by oxygen under basic conditions producing the radioactive glycolic, glyceric and some erythronic acid. Epimerization of the radioactive sugar produced the anticipated sugars. Catalytic hydrogenation of the radioactive sugar yielded a poly-ol that co-chromatographed with ribitol but not with arabitol. The importance of *ribulose biphosphate* as a universal CO₂ acceptor in a regeneration cycle was established.”

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