SEEKING FOSSIL MOLECULES

A Retrospective

by

Geoffrey Eglinton
Bristol Biogeochemistry Research Centre
University of Bristol, UK

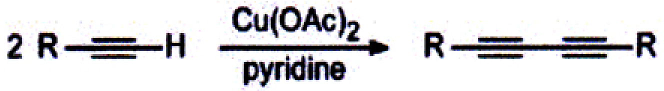
ORLEANS August 30, 2012

Early days – 1948 to 1962
Before I became an organic geochemist!

- **1948-1951 PhD Studies:** Synthesizing acetylene compounds (Manchester)

Eglinton reaction

In the related **Eglinton reaction**[4] two terminal alkynes are coupled directly by a copper(II) salt such as cupric acetate.



$$2\ R{-}{\equiv}{-}H \xrightarrow[\text{pyridine}]{Cu(OAc)_2} R{-}{\equiv}{-}{\equiv}{-}R$$

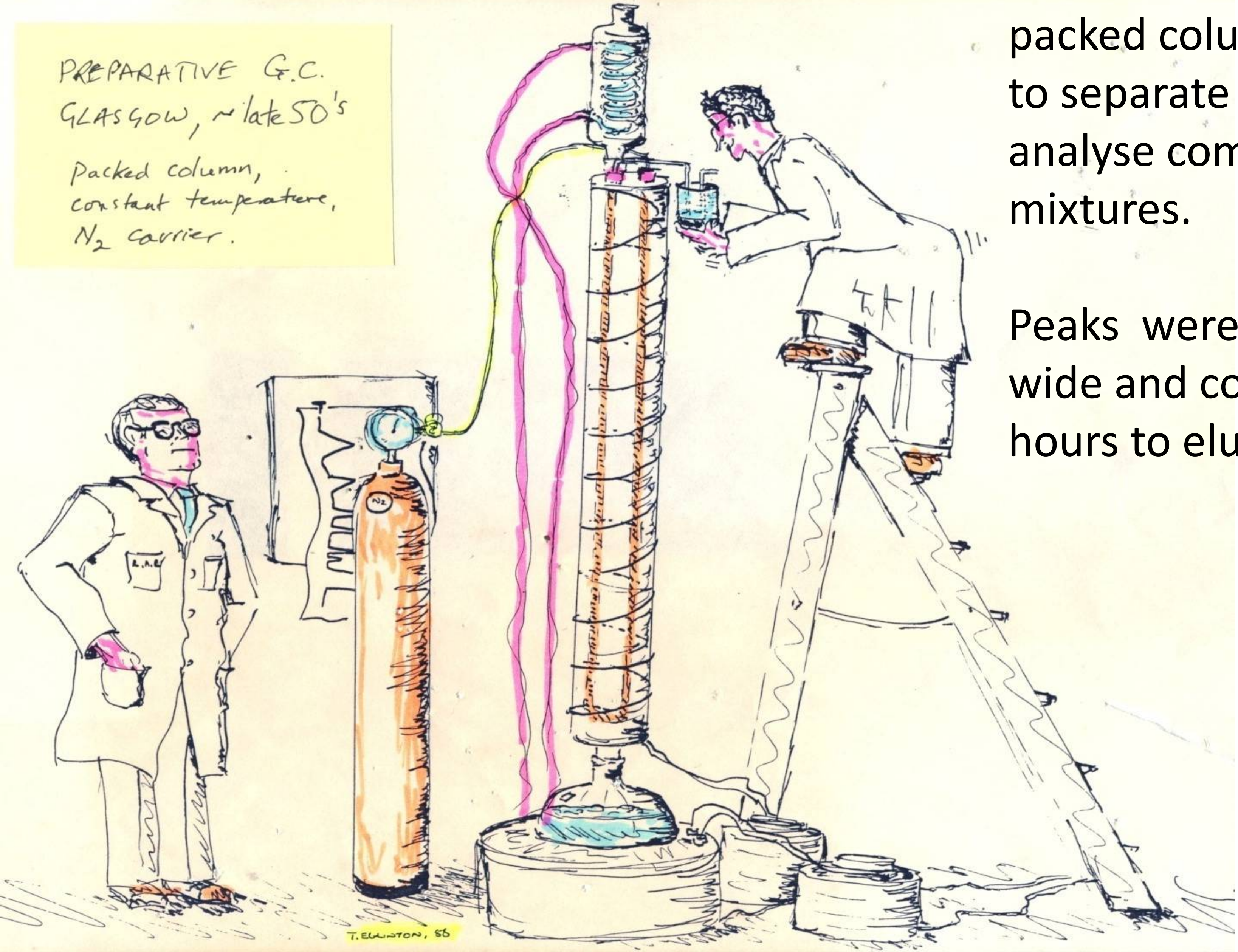
The Eglinton Reaction has been used to synthesize a number of fungal antibiotics and is important for carbon-carbon bond formation via the oxidative coupling of alkynes

- **1951- 1954** Post doc studies: Synthesis (Ohio)
  Structure determination of natural products (Liverpool)
  e.g. ergoflavin, yellow pigment of ergot fungus.

- **1954 onwards (Glasgow)**: Starting up an analytical laboratory for infra red spectroscopy (especially H-bonding studies) at University of Glasgow.

Long ago, back in the 1950's, we used packed column GC to separate and analyse complex mixtures.

Peaks were often wide and could take hours to elute.


PREPARATIVE G.C.
GLASGOW, ~ late 50's
packed column,
constant temperature,
N_2 carrier.

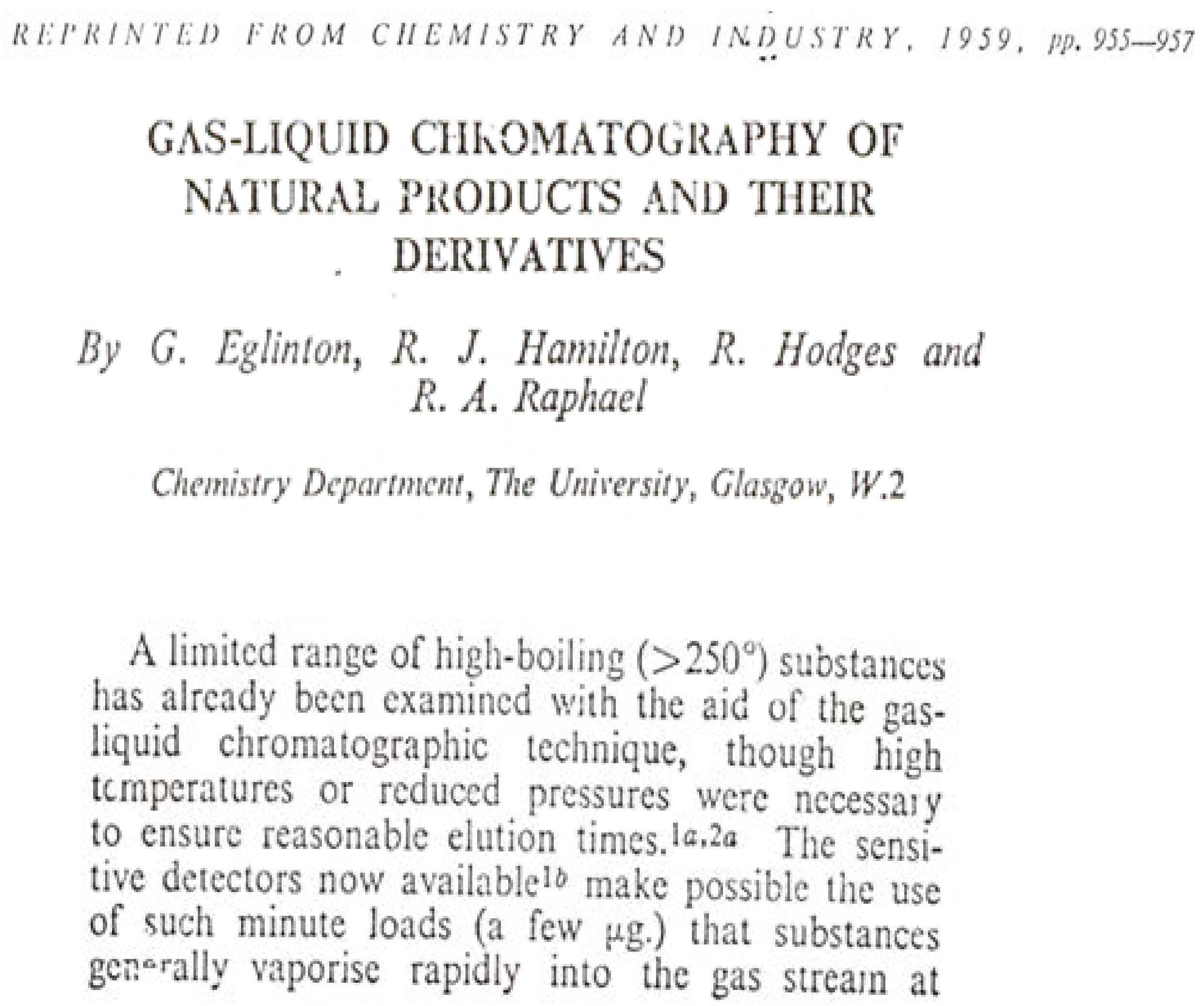
Circa 1958 (Glasgow).

Analytical GC:
This is the fabulous Pye Argon GC! The very sensitive **detector,** which was invented by James Lovelock, could work with *nanogram* quantities of sample.

At last we could attack complex mixtures, resolve closely spaced peaks and get compounds up to C30 region through in minutes – or at least a few hours!

Trimethylsilylation came along soon afterwards and revolutionised GC analysis of polar compounds. TMS derivatives of alcohols now gas chromatographed like hydrocarbons!

Example of early use of Pye-Argon GC at Glasgow: resulted in over 1000 reprint requests!



REPRINTED FROM CHEMISTRY AND INDUSTRY, 1959, pp. 955—957

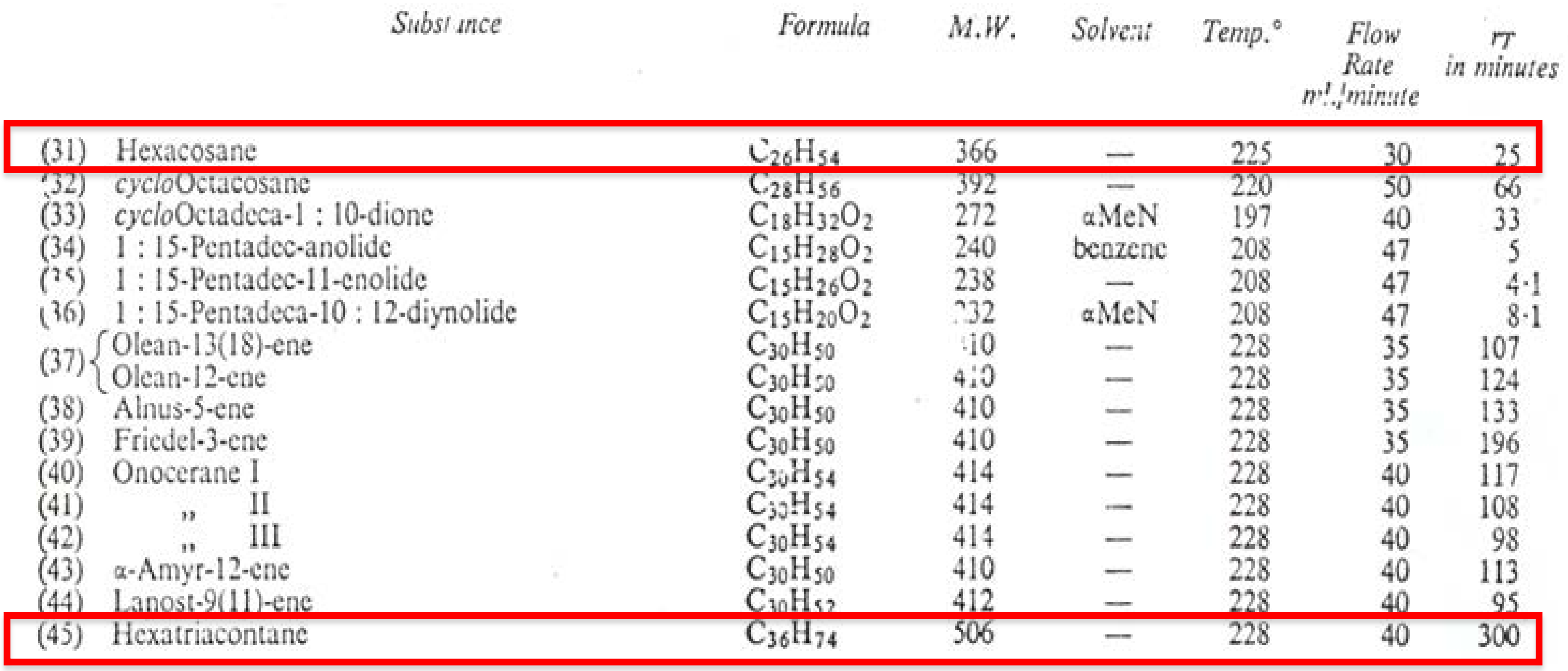
GAS-LIQUID CHROMATOGRAPHY OF NATURAL PRODUCTS AND THEIR DERIVATIVES

By *G. Eglinton, R. J. Hamilton, R. Hodges and R. A. Raphael*

Chemistry Department, The University, Glasgow, W.2

A limited range of high-boiling (>250°) substances has already been examined with the aid of the gas-liquid chromatographic technique, though high temperatures or reduced pressures were necessary to ensure reasonable elution times.[1a,2a] The sensitive detectors now available[1b] make possible the use of such minute loads (a few μg.) that substances generally vaporise rapidly into the gas stream at

Example of early use of Pye-Argon GC at Glasgow



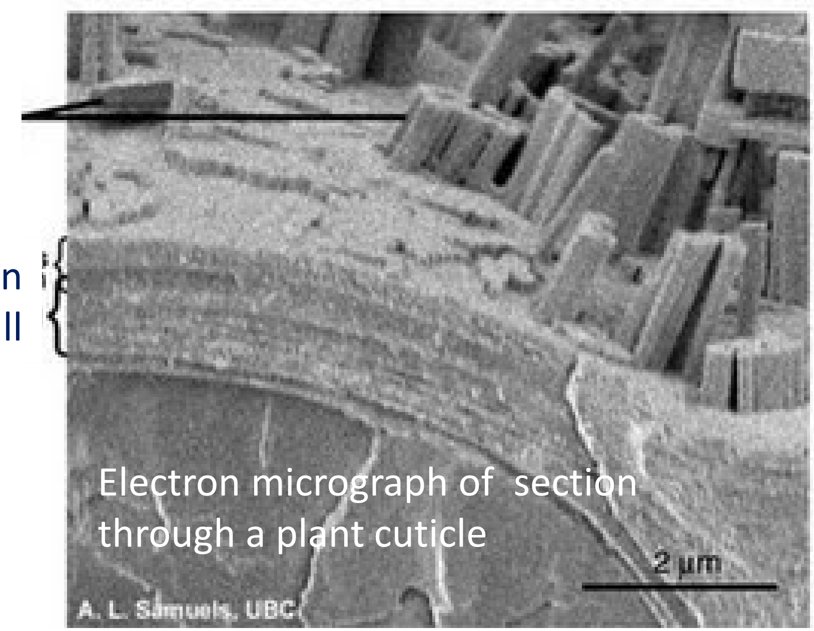
	Substance	Formula	M.W.	Solvent	Temp.°	Flow Rate ml./minute	rT in minutes
(31)	Hexacosane	$C_{26}H_{54}$	366	—	225	30	25
(32)	*cyclo*Octacosane	$C_{28}H_{56}$	392	—	220	50	66
(33)	*cyclo*Octadeca-1 : 10-dione	$C_{18}H_{32}O_2$	272	αMeN	197	40	33
(34)	1 : 15-Pentadec-anolide	$C_{15}H_{28}O_2$	240	benzene	208	47	5
(35)	1 : 15-Pentadec-11-enolide	$C_{15}H_{26}O_2$	238	—	208	47	4·1
(36)	1 : 15-Pentadeca-10 : 12-diynolide	$C_{15}H_{20}O_2$	232	αMeN	208	47	8·1
(37)	Olean-13(18)-ene	$C_{30}H_{50}$	410	—	228	35	107
	Olean-12-ene	$C_{30}H_{50}$	410	—	228	35	124
(38)	Alnus-5-ene	$C_{30}H_{50}$	410	—	228	35	133
(39)	Friedel-3-ene	$C_{30}H_{50}$	410	—	228	35	196
(40)	Onocerane I	$C_{30}H_{54}$	414	—	228	40	117
(41)	,, II	$C_{30}H_{54}$	414	—	228	40	108
(42)	,, III	$C_{30}H_{54}$	414	—	228	40	98
(43)	α-Amyr-12-ene	$C_{30}H_{50}$	410	—	228	40	113
(44)	Lanost-9(11)-ene	$C_{30}H_{52}$	412	—	228	40	95
(45)	Hexatriacontane	$C_{36}H_{74}$	506	—	228	40	300

From our viewpoint, the exciting result was that Prof. Chibnall (Cambridge Univ.) saw the paper & donated his lifetime collection of crystalline *leaf waxes* (provided in cigarette tins!).

REPRINTED FROM CHEMISTRY AND INDUSTRY, 1959, pp. 955—957

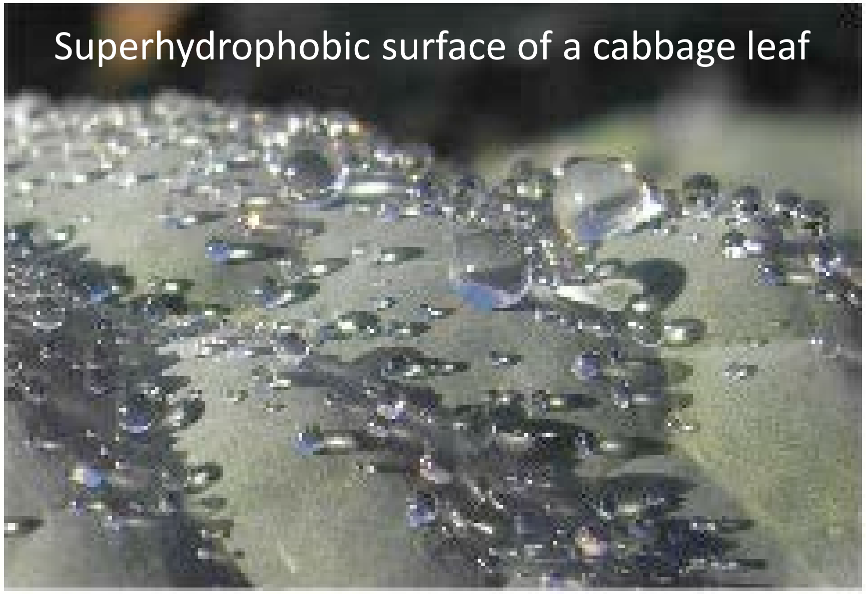
Leaf wax components

Universal crystalline coatings of vascular plants, honed by evolution for key protective and control functions.



Electron micrograph of section through a plant cuticle

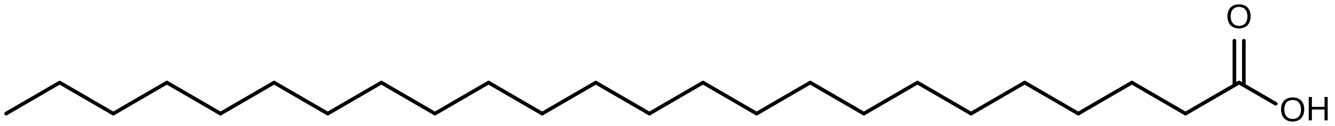
A. L. Samuels, UBC



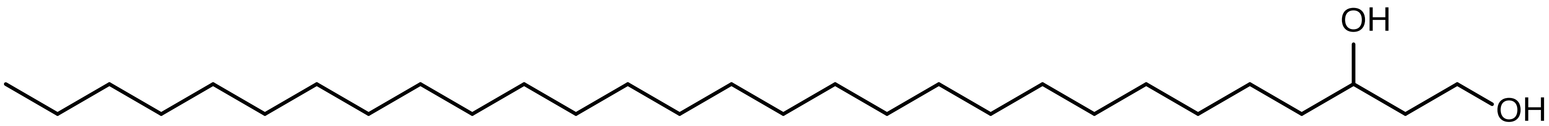
Superhydrophobic surface of a cabbage leaf

What are leaf waxes?

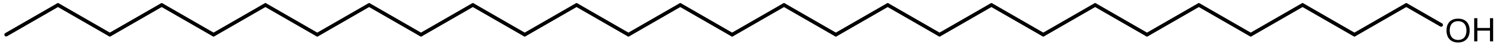
- Outer part of ‘protective skin’: protects against mechanical and microbial damage; controls water balance.
- Aliphatic hydrocarbons and derivatives: alkanes, alkanols, alkanoic acids, ketones, aldehydes, esters, etc.
- Chain length: 20 – 40 carbons (i.e. $C_{20} – C_{40}$)



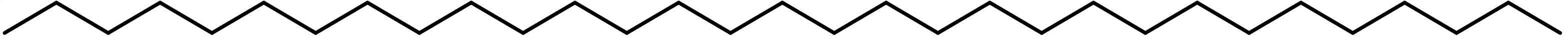
Lignoceric acid (C_{24})



Nonacosane-1,3-diol (C_{29})

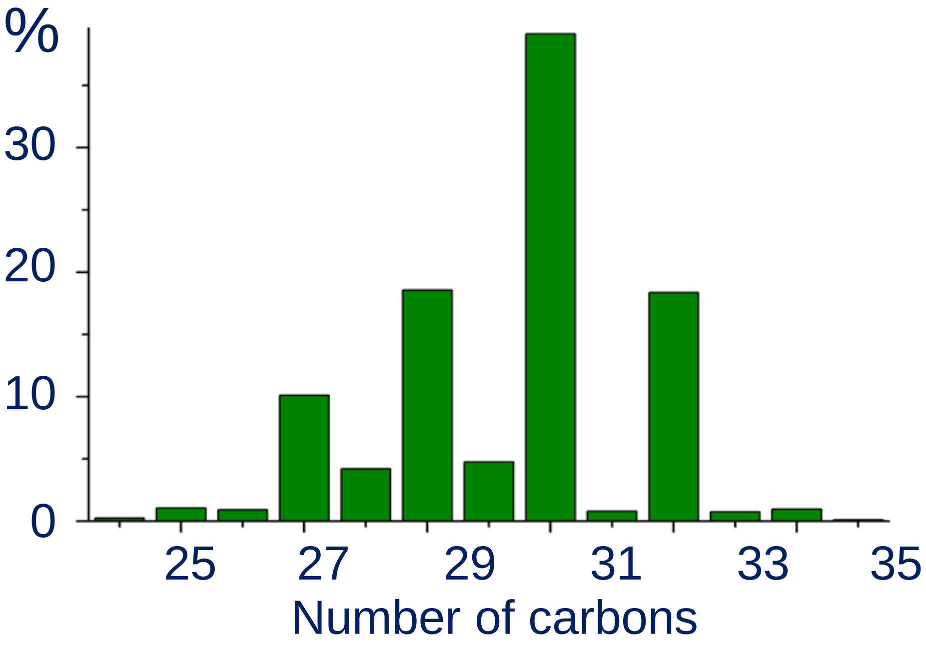


Octacosan-1-ol (C_{28})



***n*-Hentriacontane** (C_{31})

How do we display *n*-alkane data? Chain length distribution



Baobab tree (*Adansonia digitata*)

$$\mathrm{ACL}_{27\text{-}33} = 30.53$$

= **Average Chain Length** of alkanes with 27 to 33 carbon atoms

1960, Tenerife: Doing a Darwin'?

Interest in leaf wax analysis lead to my great chemotaxonomic expedition to the Canary Islands – courtesy of Carnegie Foundation!

Let us hunt plants out in the wilds and analyse them with our GC!

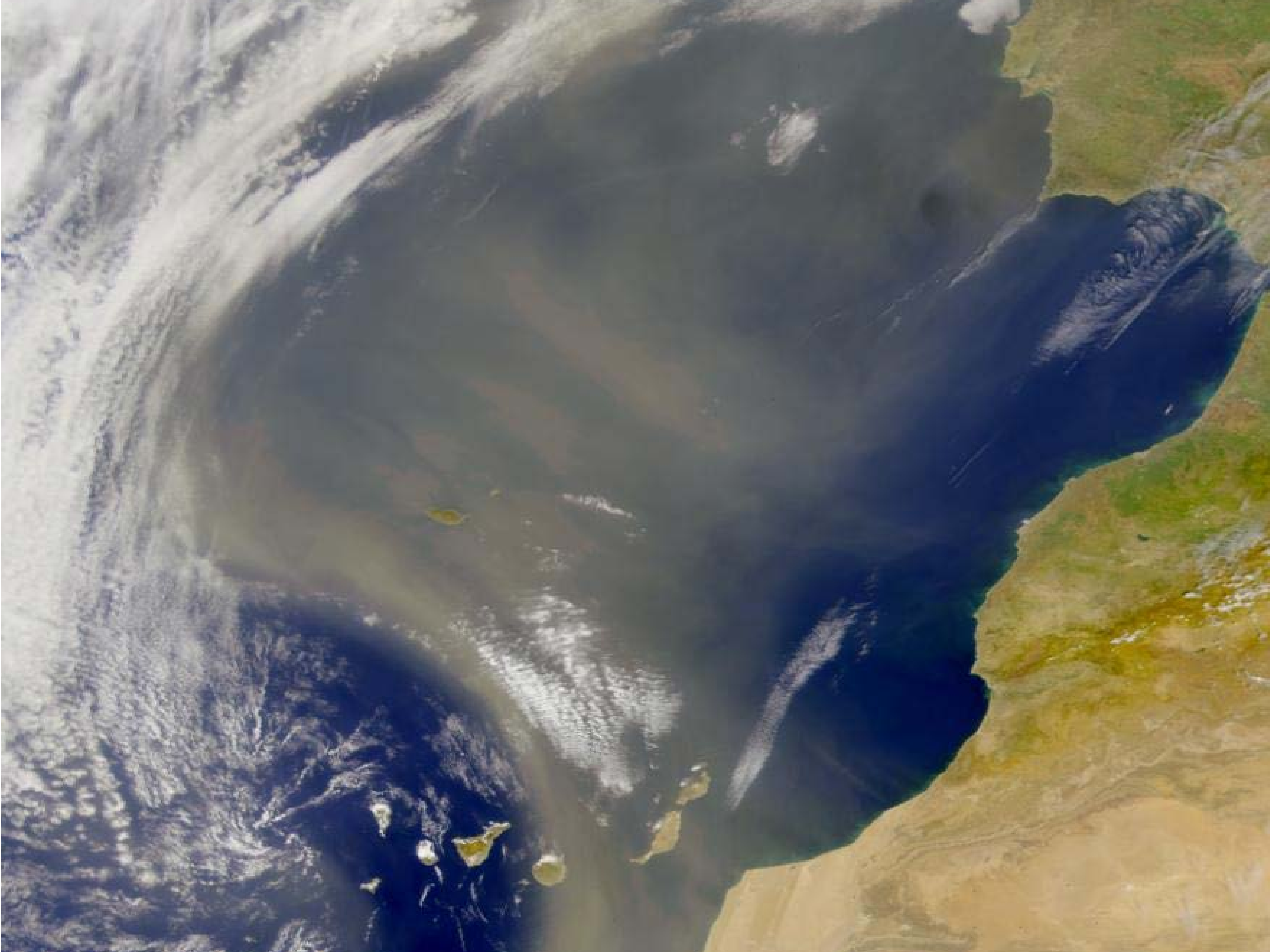
…….. Six months leave, with Ph D student, wife, two children, grandma, Pye Argon GC, IR machine and much else !

Why?: Could the leaf wax n-alkanes of related plant species show evolutionary patterns, as did the beaks of Darwin's Finches?

Where?: The Canary Islands would do fine!
Relatively young geologically (Volcanic, ca., 10Mya), colonized by plants from N. Africa, diversifying into a variety of microclimates.

Resources?: A widespread, wax-rich, plant Family (the Crassulaceae), resident expert botanist (Dr Sventenius), Natural Products Chemist host (Prof Antonio Gonzalez Gonzalez) and his laboratory (Universidad de La Laguna) and transport (the Professorial white Jaguar). Also College accommodation and maid!

AND what a climate!



The Fortunate Isles!

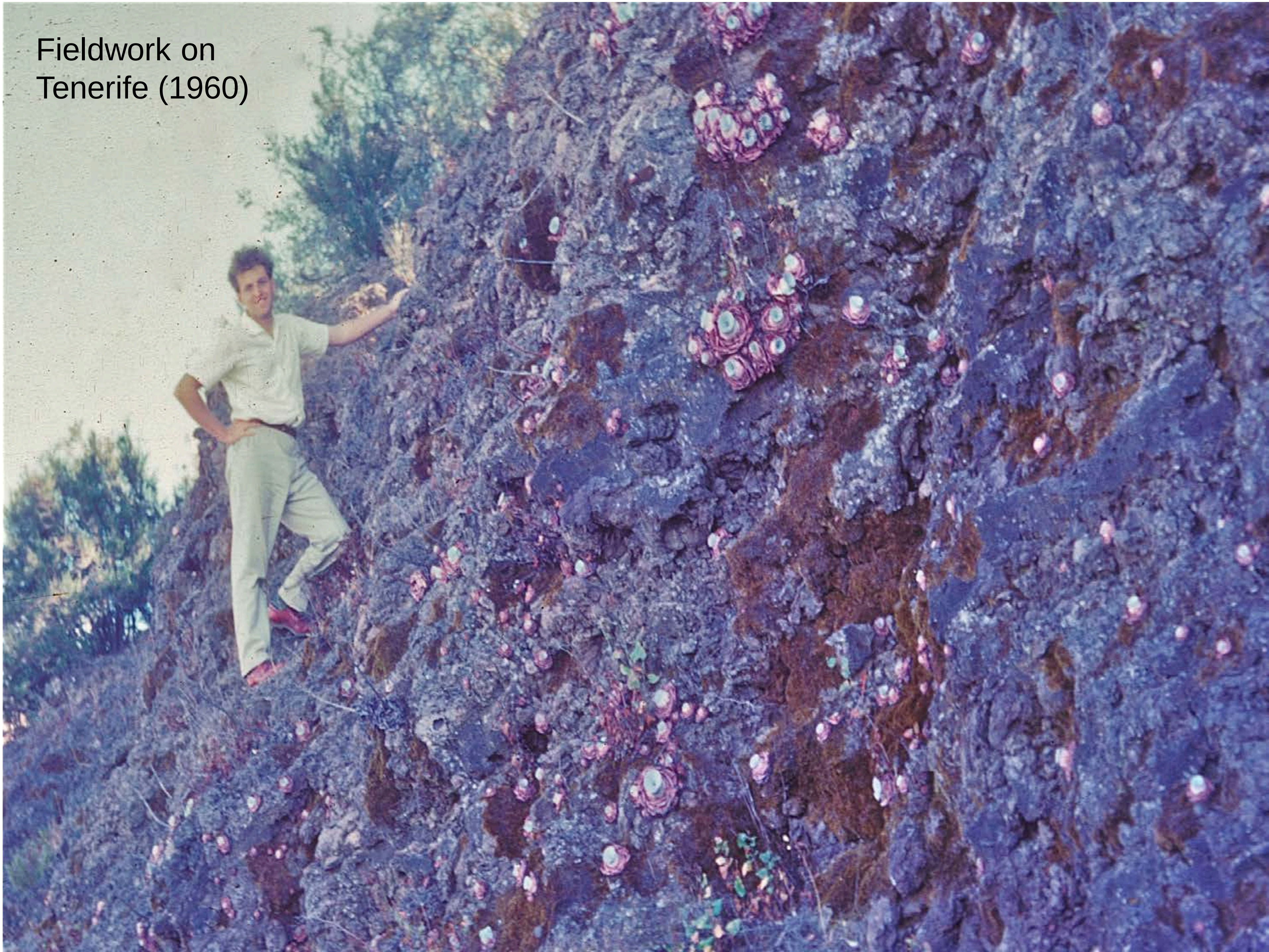
Pico Teide from village of
Preece on Tenerife,1960

Note for 2013:
26th International meeting in
Organic Geochemistry
will take place on
Tenerife, Canary Islands, Spain

September 15th - 20th 2013

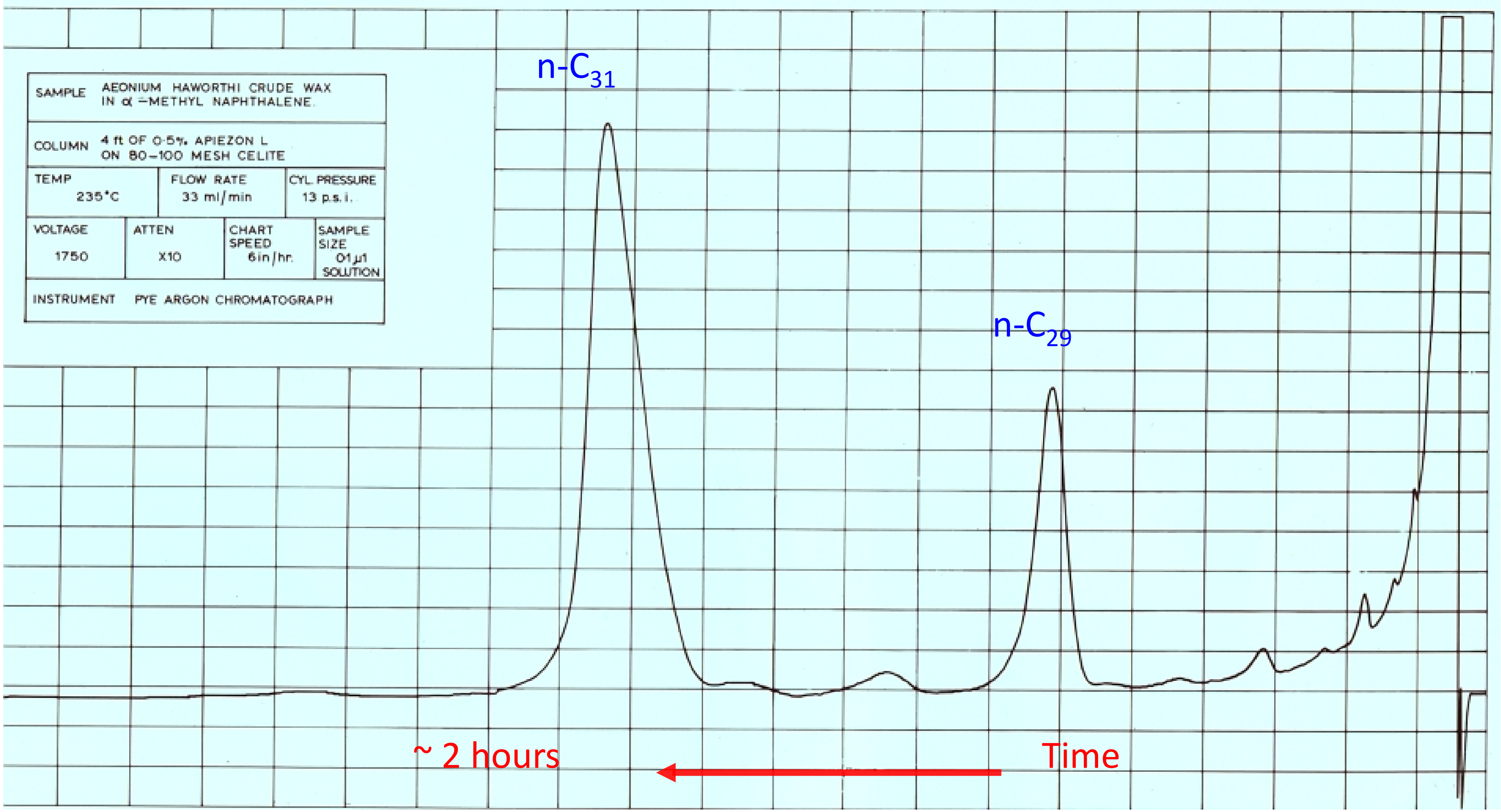
University of La Laguna, Tenerife (1960)

Fieldwork on
Tenerife (1960)



Documenting plant specimens for Kew Gardens, London

Alkanes of *Aeonium haworthii* (Crassulaceae)



SAMPLE AEONIUM HAWORTHI CRUDE WAX IN α-METHYL NAPHTHALENE.

COLUMN 4 ft OF 0·5% APIEZON L ON 80–100 MESH CELITE

TEMP 235°C

FLOW RATE 33 ml/min

CYL. PRESSURE 13 p.s.i.

VOLTAGE 1750

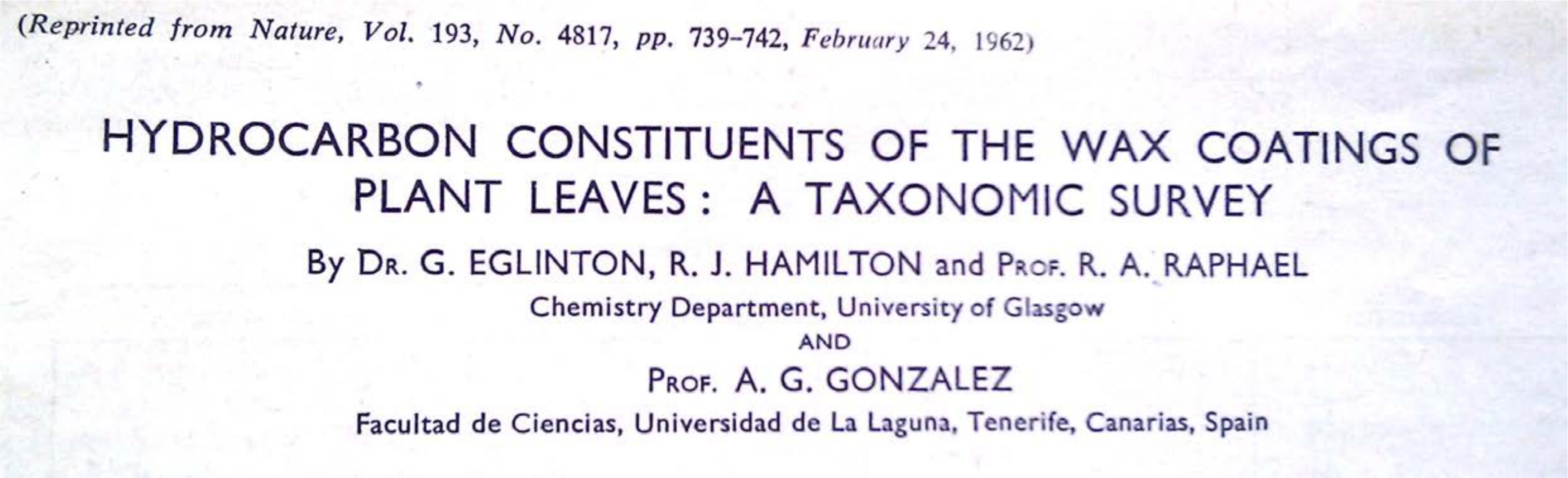
ATTEN X10

CHART SPEED 6in/hr.

SAMPLE SIZE 0·1 µl SOLUTION

INSTRUMENT PYE ARGON CHROMATOGRAPH

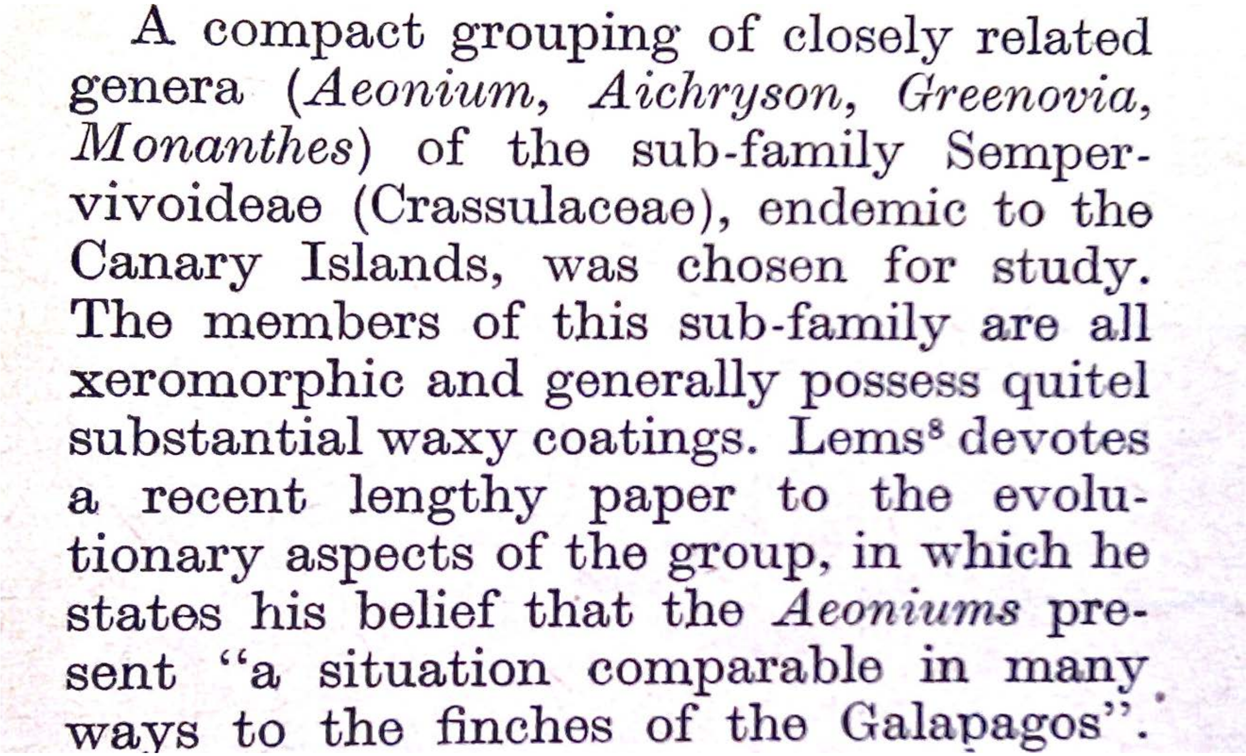
C_{36} ~ 5 hours!



(*Reprinted from Nature, Vol.* 193, *No.* 4817, *pp.* 739–742, *February* 24, 1962)

HYDROCARBON CONSTITUENTS OF THE WAX COATINGS OF PLANT LEAVES: A TAXONOMIC SURVEY

By DR. G. EGLINTON, R. J. HAMILTON and PROF. R. A. RAPHAEL
Chemistry Department, University of Glasgow
AND
PROF. A. G. GONZALEZ
Facultad de Ciencias, Universidad de La Laguna, Tenerife, Canarias, Spain



A compact grouping of closely related genera (*Aeonium*, *Aichryson*, *Greenovia*, *Monanthes*) of the sub-family Sempervivoideae (Crassulaceae), endemic to the Canary Islands, was chosen for study. The members of this sub-family are all xeromorphic and generally possess quitel substantial waxy coatings. Lems[8] devotes a recent lengthy paper to the evolutionary aspects of the group, in which he states his belief that the *Aeoniums* present "a situation comparable in many ways to the finches of the Galapagos".

Circa 1962 (Glasgow): Molecular organic geochemistry – I take the plunge!

Why?:

- Papers on natural products, synthesis and IR were getting too numerous for my liking!

- It seemed time to find a new , little-explored research area - with few papers to read through!

- Would be good if I could use the new topic to escape the lab. and explore the ‘real’ world -as did biologists and geologists.

So, what topic looked promising?

Back in 1948, I had wondered about getting molecular signals out of some fossil organic matter, but was advised to 'get on with my B.Sc.'. The stuff which had taken my fancy was a stinking , black, sticky 'goo' oozing out of a limestone cliff in Derbyshire and which went by the impressive name of **Elaterite**!

But this was really too messy to start with!

Analysing rocks and fossils for traces of remnant biological compounds, like chlorophyll, sounded fun and challenging.

But, was the time right?

- Possibly - because powerful, newly -available techniques, like GC, IR and MS should help make a start.

- And there was a small body of interesting old literature:
  - for example, a stimulating lecture by Sir Robert Robinson on "A Dual Origin for Petroleum?" .
  - And deep in the old German literature there were papers by Treibs on fossil porphyrin pigments (which I did not find out about when I started - *mea culpa* !).

In the beginning…well, 1938!

“An organic solvent extract of a bituminous oil shale showed a magnificent red color and the characteristic absorption spectrum of the metal complexes of pyrrole pigments. . . . The appearance of a preservation of complicated pigment materials through geological eras permits hope that the same may also be true of other types of molecules ”.

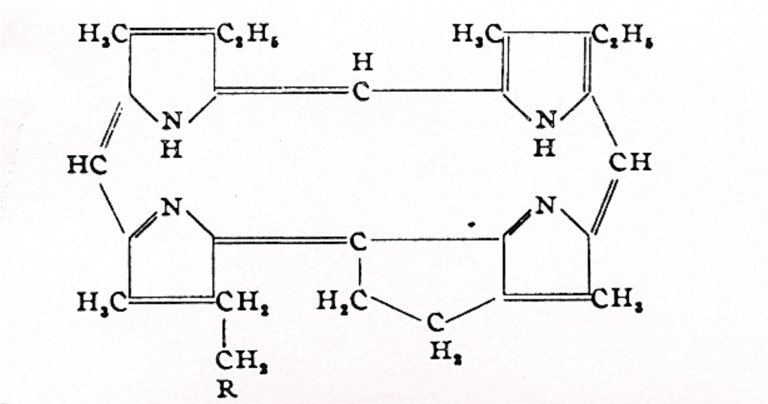
- Alfred Treibs

Our pioneering founder of Molecular Organic Geochemistry, **Alfred Treibs**, sits besuited and suitably serious in the front row of the **1970 G.R. Conference** photograph. He showed us that the molecular structures of our beloved biomarkers are the keys to understanding organic geochemical provenances and processes.

The Famous Treibs' Scheme

Treibs' Scheme links chlorophyll to the range of porphyrins and metalloporphyrins that he and his students had isolated from petroleums and shales. It was first published in **1934** in *Annalen.*

The structure of the main product, **deoxophylloerythroetioporphyrin (DPEP)**, at first sight looks pretty weird and strangely angular but this is not a problem deriving from lack of understanding, but simply the limitations of the printers' craft at the time.



R = H. Desoxophyllerythro-ätioporphyrin.

Anyway, the organic reactions he proposed, leading from chlorophyll to DPEP, still make excellent sense today. *See Baker and Louda, "The Legacy of the Treibs Samples," pages 3-128 in The Treibs' Centenary Volume, Munich 1999.*

And another thing I did not find out about at the time (poor excuse, but no Googling then!):

The **Gordon Research Conference, August 19th, 1963 (49 years ago!)**

Quote from John Hunt's plenary GRC presentation notes on:
"Origin of Petroleum – Basic Geochemical Concepts"

"In the last few years there have been some remarkable advances in understanding the origin of petroleum. The primary reason for this is a ***rapid advance in analytical procedures*** which has made it possible to define in some detail the distribution of petroleum and other types of organic matter in both recent and ancient sediments. ***As a result we have been accumulating data on the origin of petroleum during the last five or ten years, as opposed to simply accumulating theories during the previous decades".***

1963 (Berkeley, California): Starting up Organic Geochemistry!

So, in 1963 -1964 I took a one year leave of absence from Glasgow, and went West to California in search of answers to the questions:-

"Just what molecular records does life leave in the Earth's crust and how far back in time can we trace them?"

- I set up my first dedicated O.G. Facility in Nobel laureate Melvin Calvin's lab in the Chemistry Dept., at Berkeley, University of California.
- Plenty $ and completely empty lab available, also a state of the art probe mass spectrometer and resident expert (Al Burlingame), and the new Aerograph GC with *programmable temperature oven*.
- We were in business! But Calvin wanted to analyze the oldest rocks for chemical signs of early life. I wanted to start with very young rocks—so we compromised on *the 50 million yr old* ***Green River Shale****!*
- N.B. it was '**flower power**' time in San Francisco - very distracting!

A HAPPY HUNTING GROUND FOR BIOMARKERS!- **THE GREEN RIVER SHALE, WYOMING**

Thick sequences of Eocene (50Mya) sediments ,deposited in a vast area of inter-montane, shallow anoxic lakes.

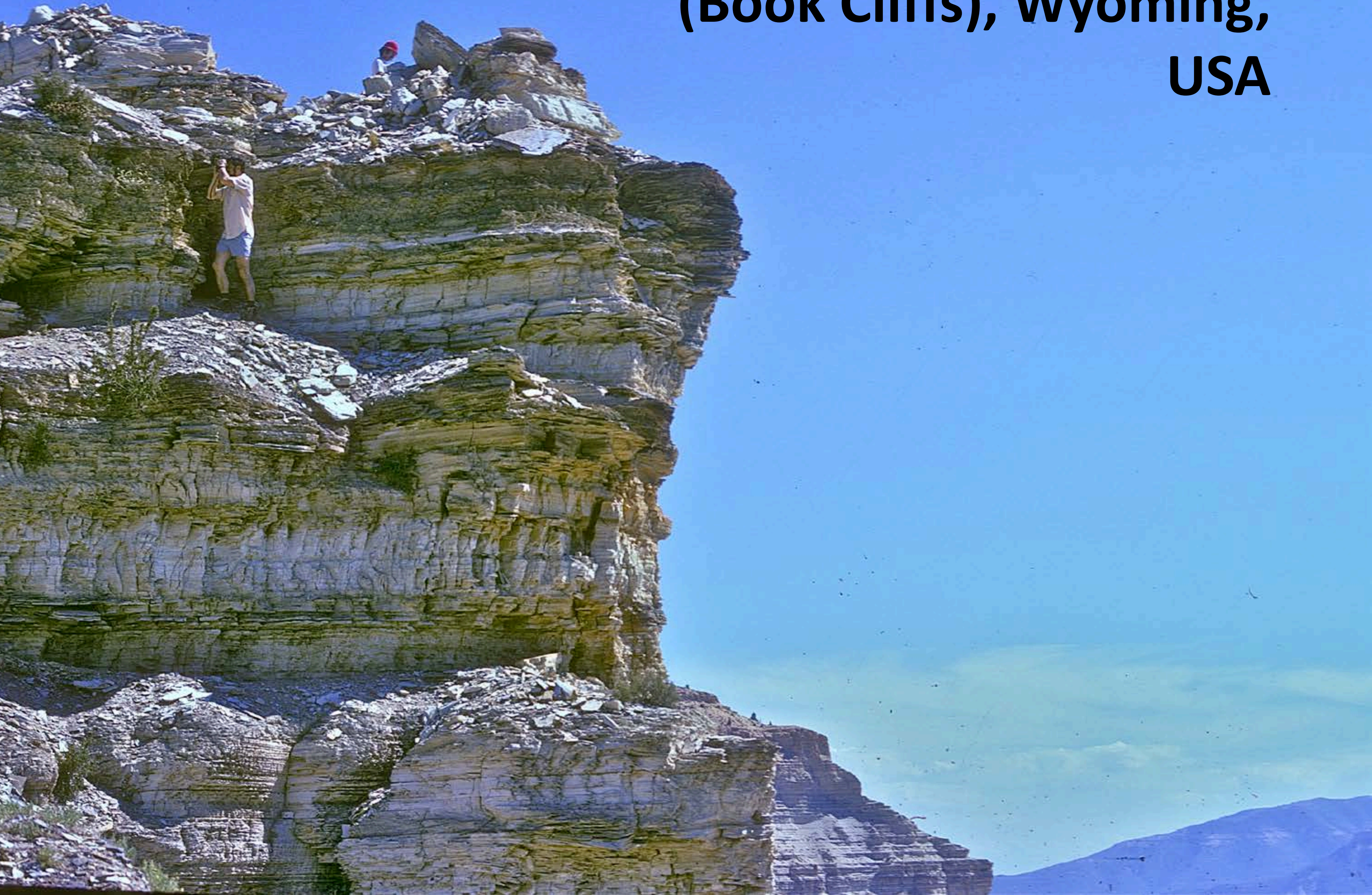
WHY STUDY?:

- Largest oil shale deposit in world. Also some bitumen veins-Gilsonite
- Fossiliferous (plant and animal), organic –rich ,finely laminated, carbonate-rich shales.
- Likely to contain original bio-organic compounds: -alkanes, alcohols, acids, pigments etc- gives coloured solvent extracts.

Should be excellent proving ground for analytical techniques and theories of biomarker origins.

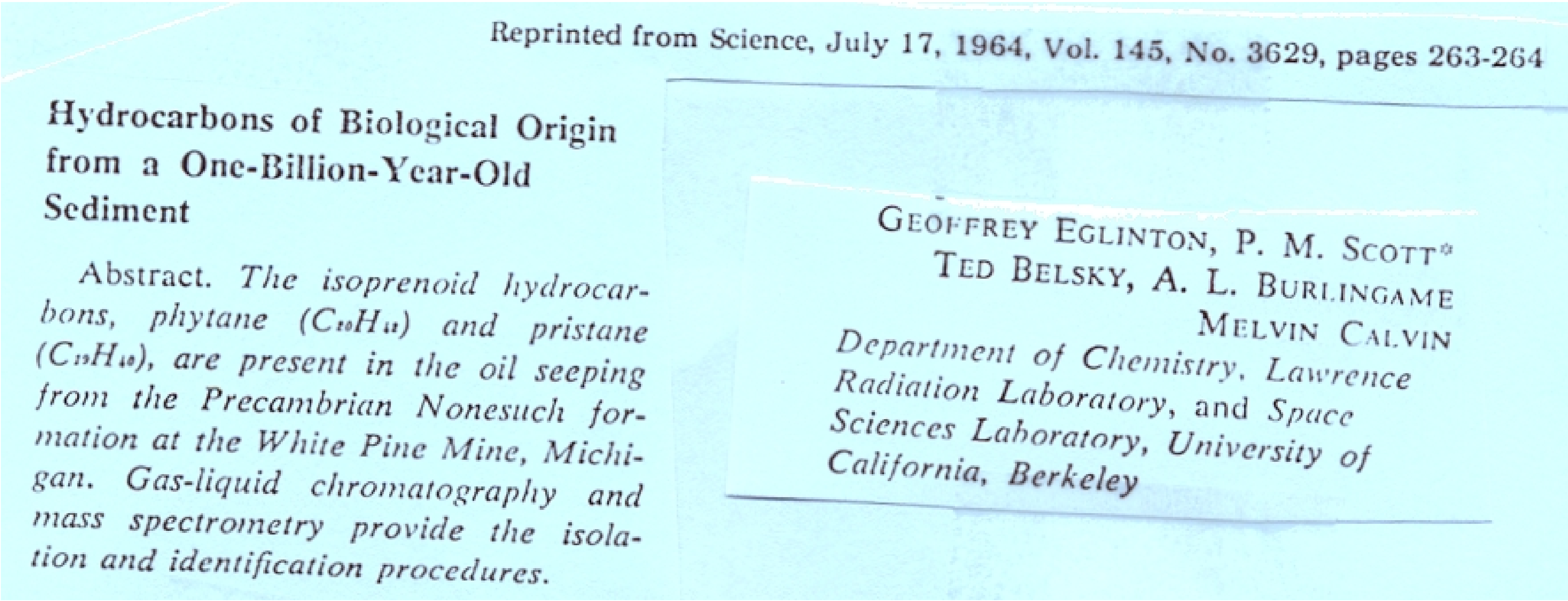
And a great place to sample **- IN THE WILD WEST AND JOHN WAYNE COUNTRY!**

The Green River Shale (Book Cliffs), Wyoming, USA



The Green River Shale proved a good practice ground, giving us abundant pristane, phytane, etc….

So we moved onto one of Calvin’s pet Pre-Cambrian rocks from the Nonesuch Fm., Michigan USA



Reprinted from Science, July 17, 1964, Vol. 145, No. 3629, pages 263-264

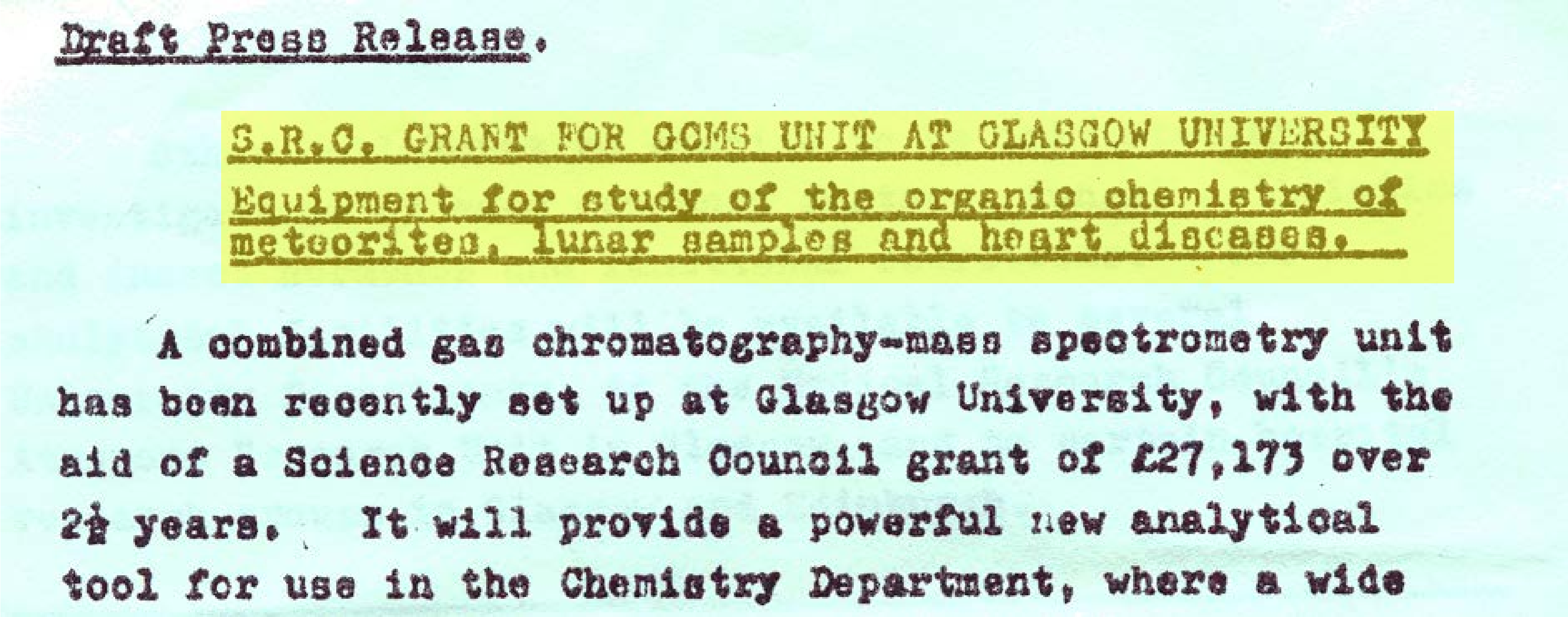
Hydrocarbons of Biological Origin from a One-Billion-Year-Old Sediment

Abstract. *The isoprenoid hydrocarbons, phytane ($C_{20}H_{42}$) and pristane ($C_{19}H_{40}$), are present in the oil seeping from the Precambrian Nonesuch formation at the White Pine Mine, Michigan. Gas-liquid chromatography and mass spectrometry provide the isolation and identification procedures.*

GEOFFREY EGLINTON, P. M. SCOTT[*]
TED BELSKY, A. L. BURLINGAME
MELVIN CALVIN
Department of Chemistry, Lawrence Radiation Laboratory, and Space Sciences Laboratory, University of California, Berkeley

1964: Return to Glasgow and our own organic geochemistry facility.

Meanwhile, our SRC proposal for the 1st GC-MS in a UK university had been successful.



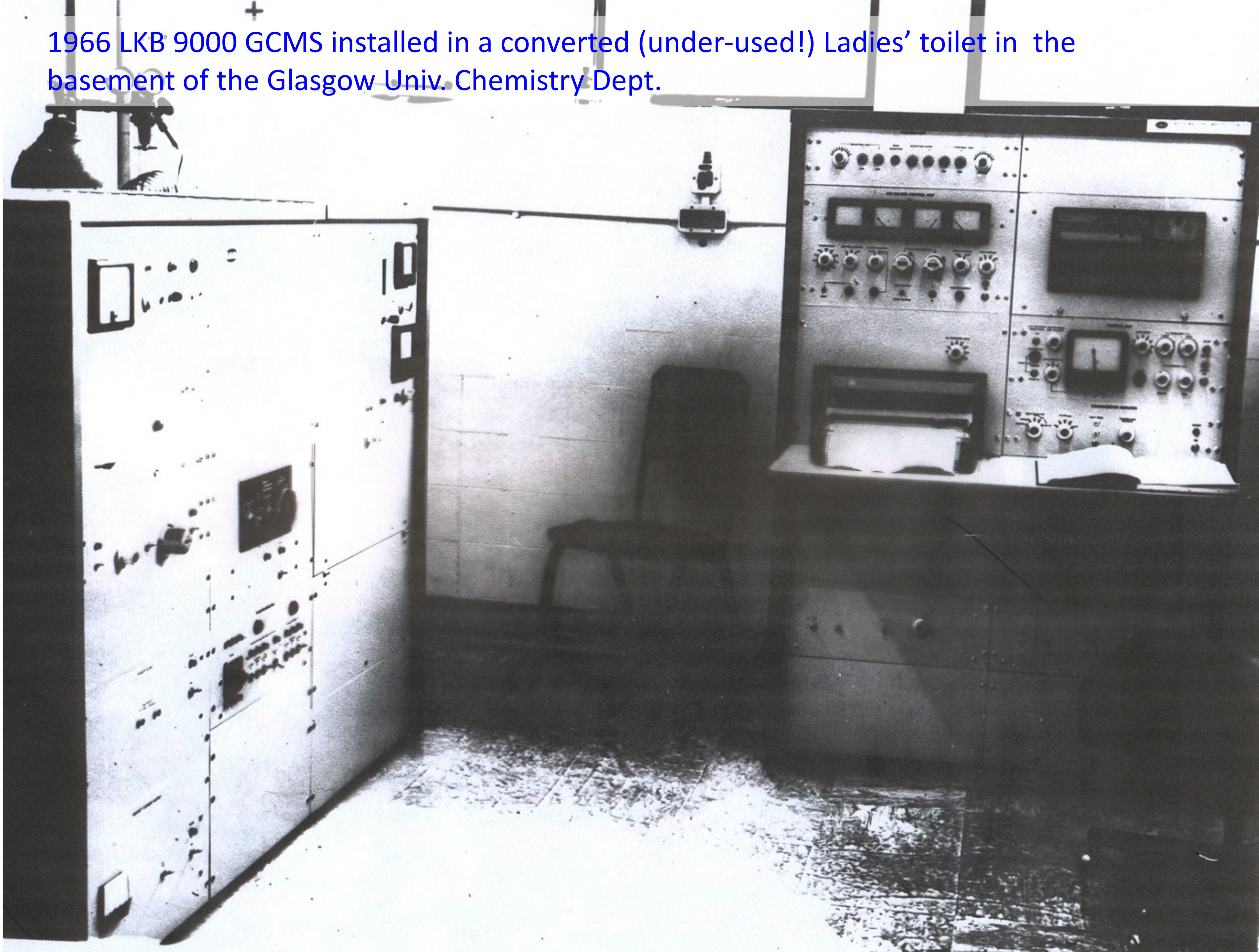
Draft Press Release.

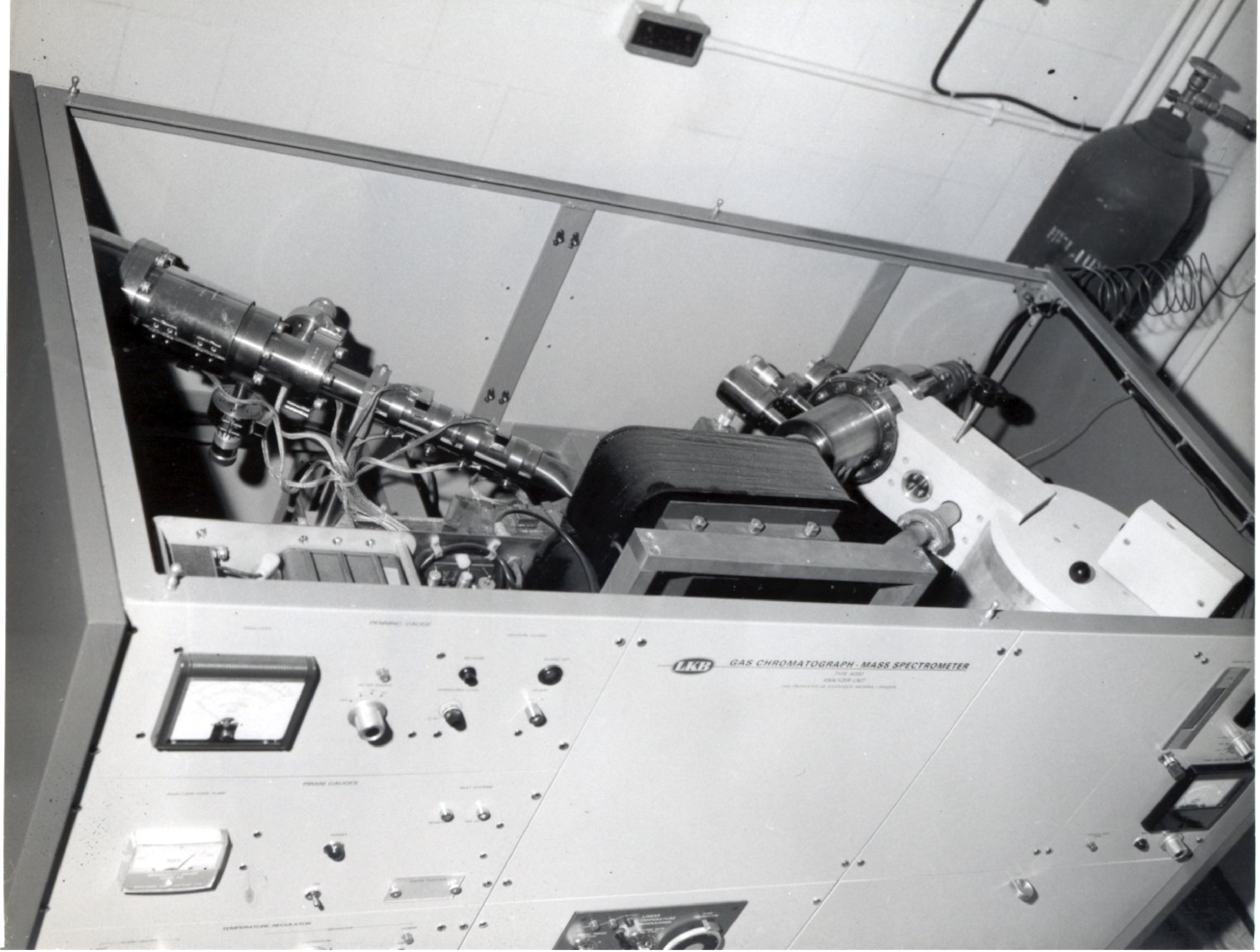
S.R.C. GRANT FOR GCMS UNIT AT GLASGOW UNIVERSITY

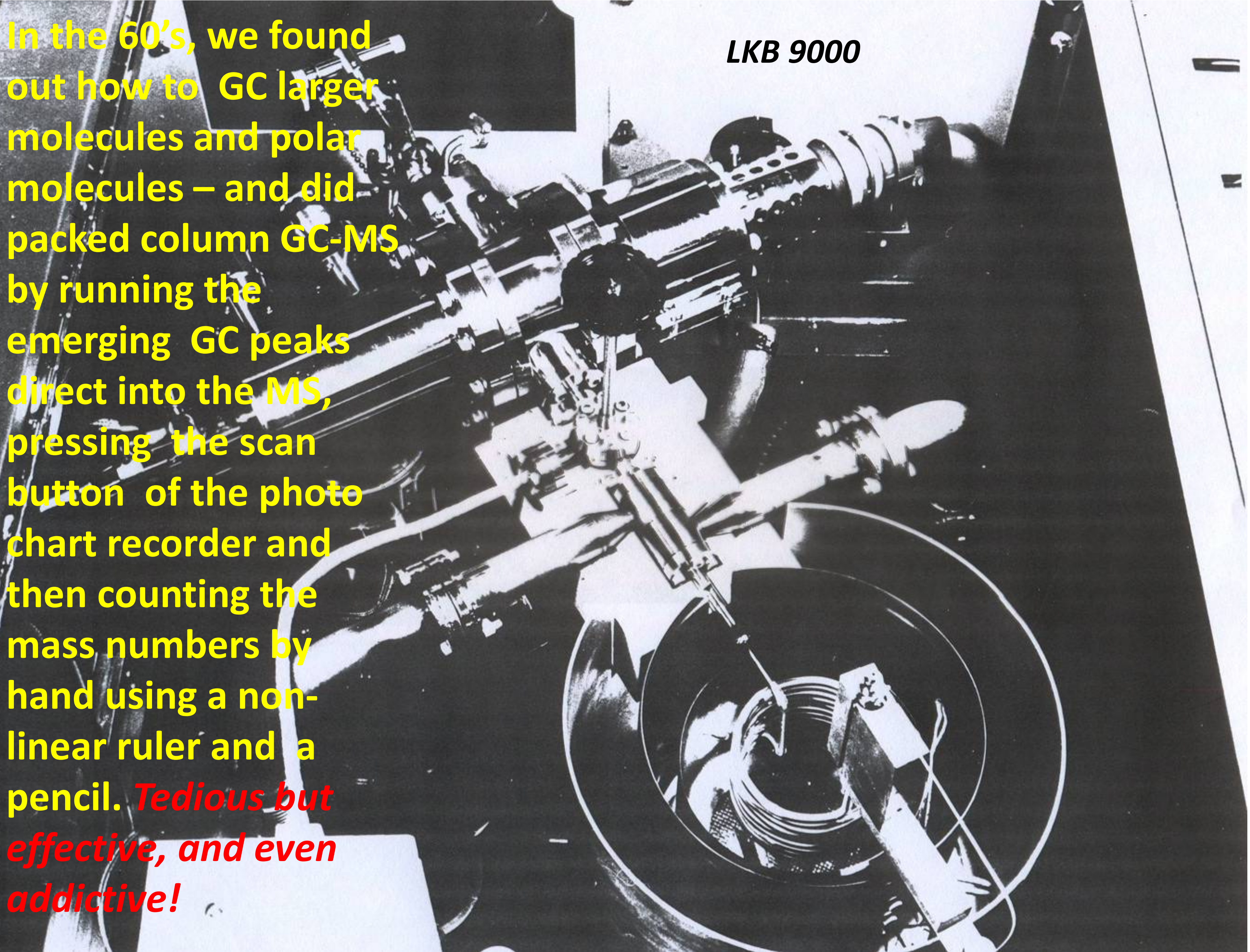
Equipment for study of the organic chemistry of meteorites, lunar samples and heart diseases.

A combined gas chromatography-mass spectrometry unit has been recently set up at Glasgow University, with the aid of a Science Research Council grant of £27,173 over 2½ years. It will provide a powerful new analytical tool for use in the Chemistry Department, where a wide

1966 LKB 9000 GCMS installed in a converted (under-used!) Ladies' toilet in the basement of the Glasgow Univ. Chemistry Dept.



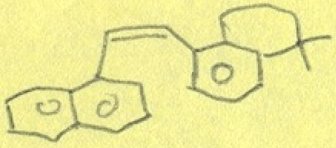

PENNING GAUGE
LKB
GAS CHROMATOGRAPH - MASS SPECTROMETER
PIRANI GAUGES
TEMPERATURE REGULATOR



LKB 9000

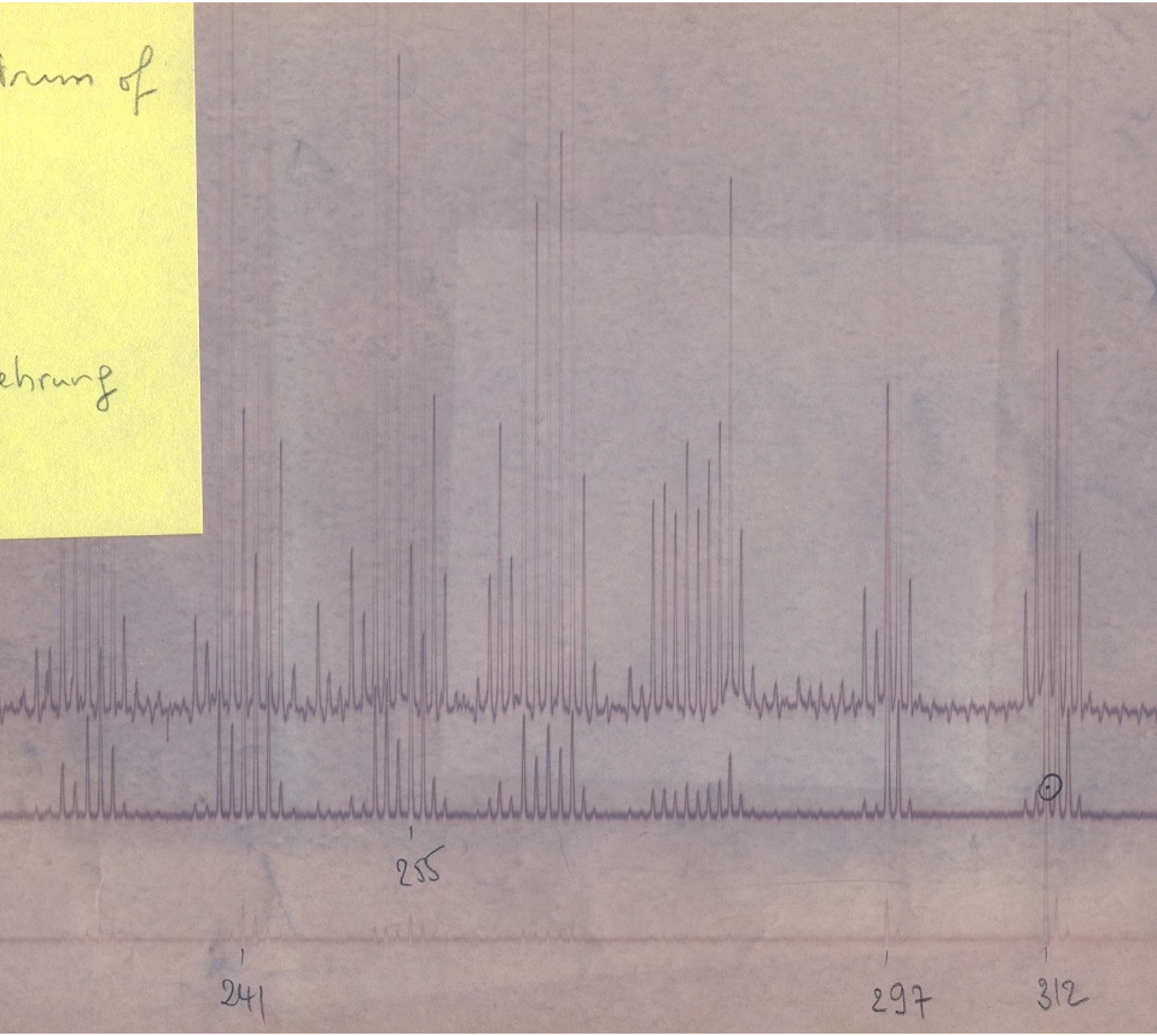
In the 60's, we found out how to GC larger molecules and polar molecules – and did packed column GC-MS by running the emerging GC peaks direct into the MS, pressing the scan button of the photo chart recorder and then counting the mass numbers by hand using a non-linear ruler and a pencil. ***Tedious but effective, and even addictive!***

1979 Mass spectrum of



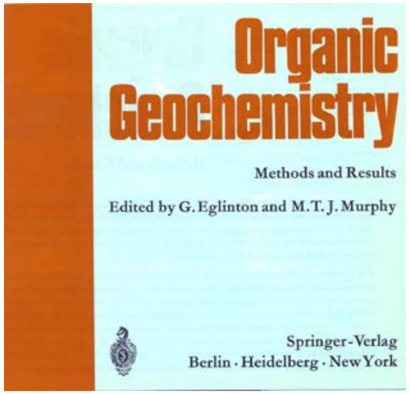
from Patrick Wehrung

Strasbourg



1966-1968: Organic Geochemistry at Glasgow

1966: Sister (Dr) Mary Murphy ,“first nun on the moon”, arrives and agrees to co-edit planned *“Organic Geochemistry Methods and Results” book* for Springer-Verlag.



Organic Geochemistry

Methods and Results

Edited by G. Eglinton and M. T. J. Murphy

Springer-Verlag
Berlin · Heidelberg · New York

1966: Alfred Treibs (“father of Organic Geochemistry”) visits.

Mid-60s: Pierre Albrecht (Strasbourg) joins group for a while.

1968: PhD Student Bill Henderson surreptitiously - but successfully! – converts packed column LKB to *capillary GC/MS*. Steroidal and triterpenoidal hydrocarbons in Green River Shale reported (*Chem. Comm.)*

1968: Capillary GC-MS gave us lots of sharp, well-resolved GC peaks with clean mass spectra.

Biomarker hunts became the in-thing.

'Another day, another biomarker', as Ollie Zafiriou put it!

Identification of Steroids and Triterpenes from a Geological Source by Capillary Gas–liquid Chromatography and Mass Spectrometry

By W. HENDERSON, V. WOLLRAB, and G. EGLINTON*
(*Organic Geochemistry Unit, School of Chemistry, Bristol University*)

(1968)

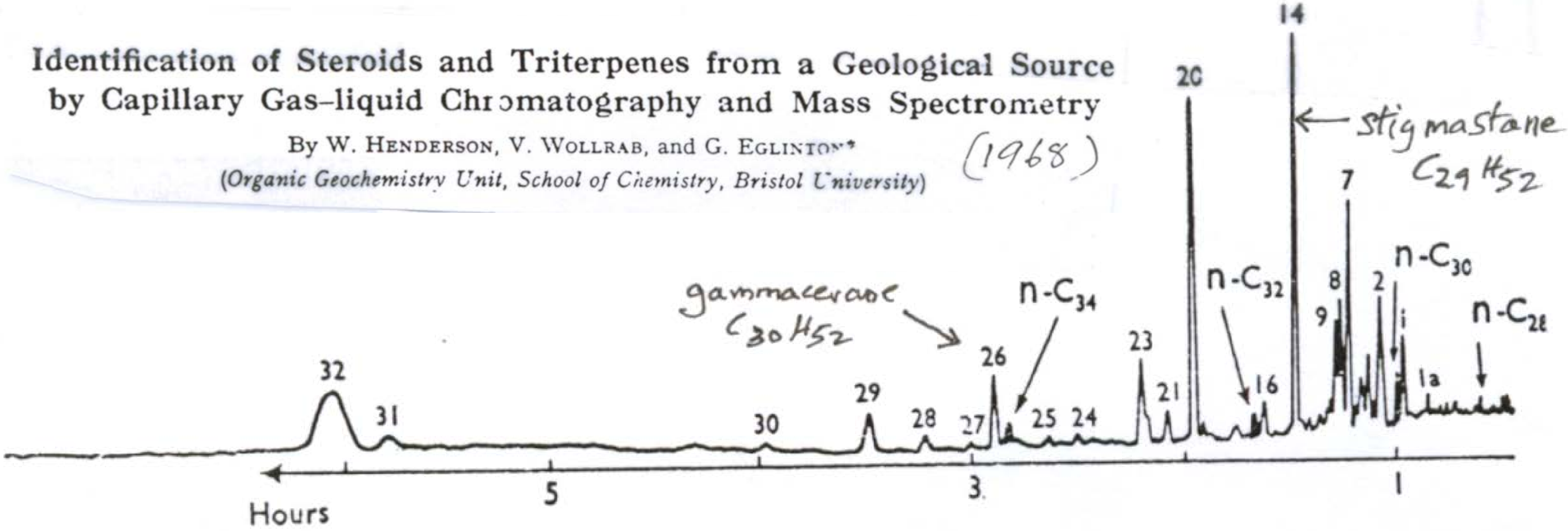


FIGURE 1. *Portion of the g.l.c. of the total branched and‡ cyclic alkane fraction from the Green River shale with the reference* n-C_{28}, n-C_{30}, n-C_{32}, and n-C_{34} *alkanes added. Conditions:* 200 ft. × 0·01 in. *stainless steel capillary column coated with Apiezon L grease (AP-L),* 250°, *helium flow rate* 2 *ml./min.*

‡ LKB 9000 at the Chemistry Department, University of Glasgow. We thank Dr. C. J. W. Brooks for the use of this instrument.

Correlation of Stereoisomerism in Present Day and Geologically Ancient Isoprenoid Fatty Acids

(1968)

by

IAIN MACLEAN
GEOFFREY EGLINTON*
K. DOURAGHI-ZADEH†
Department of Chemistry,
University of Glasgow

R. G. ACKMAN
S. N. HOOPER
Fisheries Research Board of Canada,
Halifax Laboratory,
Halifax, Nova Scotia

High resolution gas chromatography of the methyl esters of individual isoprenoid fatty acids isolated from a geological source of the Eocene period has shown a diastereoisomer composition compatible with a chlorophyll derivation for the acids. The L-menthyl esters give enhanced separation of isoprenoid acid diastereoisomers on an optically inactive GC substrate and could be employed on samples with more complex stereochemistry.

(*Reprinted from Nature*, *Vol.* 218, *No.* 5146, *pp.* 1019–1023, *June* 15, 1968)

1968: Invited to move to the University of Bristol

Setting up the Organic Geochemistry Unit (OGU) in the School of Chemistry, with James Maxwell.

- Office and laboratory space available.
- Some equipment moved from Glasgow, but GC-MS had to stay there!

So, we ask for the moon!

A SAMPLE OF MOON DUST, PLEASE!

WHY WERE WE ASKING FOR THE MOON?

BECAUSE IT WAS ON SPECIAL OFFER!

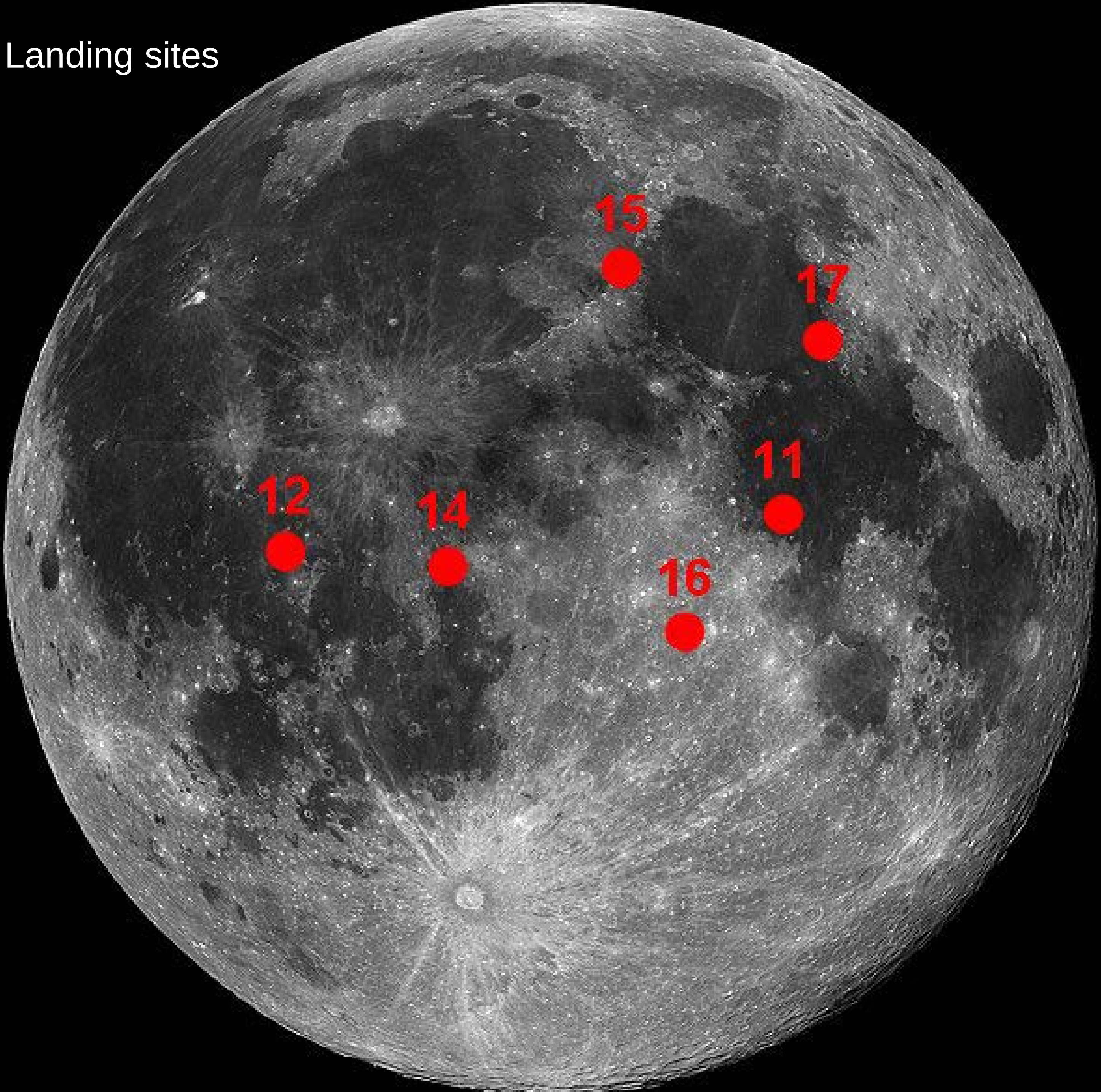
- NASA had invited Proposals from US and International Scientists as to what science we could do with any samples of lunar soil or rocks to be brought back by the planned Apollo Missions

- We proposed to look for traces of **biomarker hydrocarbons**–and hence evidence of past life- using gas chromatography and mass spectrometry. ‘Clean Room’ methodology would be essential for keeping out earthly contaminants- fingerprint and dandruff lipids, lubricants….ugh.

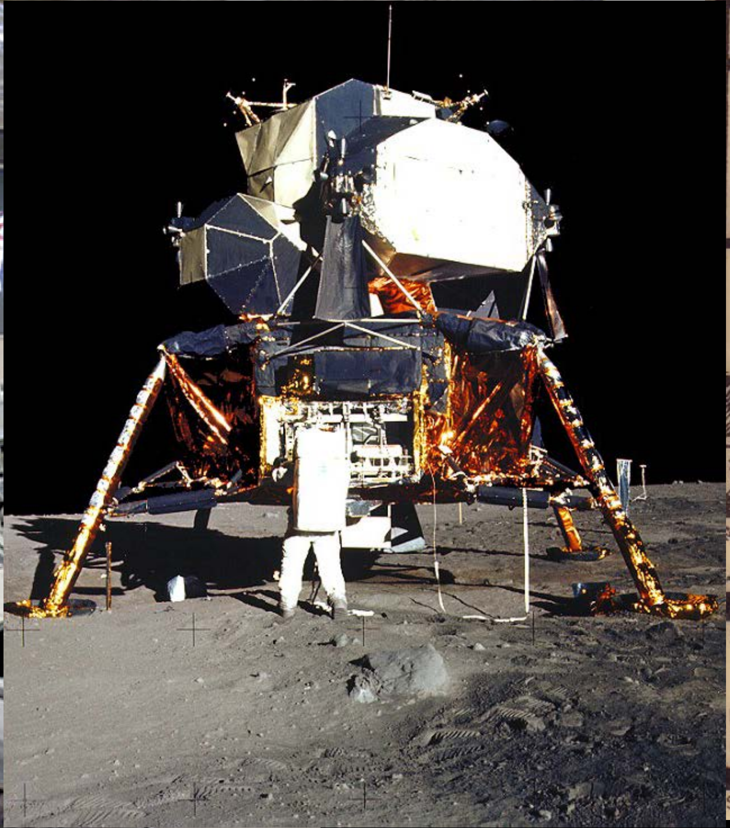
- Our proposal had been approved. We had the techniques, -but, in moving from Glasgow to Bristol we had had to leave our GC-MS behind.

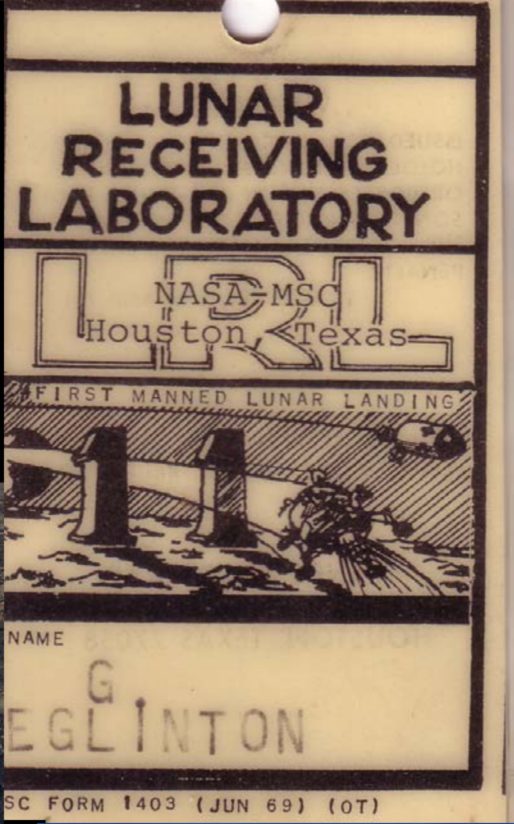
So the moon was one very good reason for giving us sparklingly-clean laboratories and a new GC- MS!

Apollo Landing sites



1969: Organic Geochemistry goes to the Moon





LUNAR RECEIVING LABORATORY

LRL

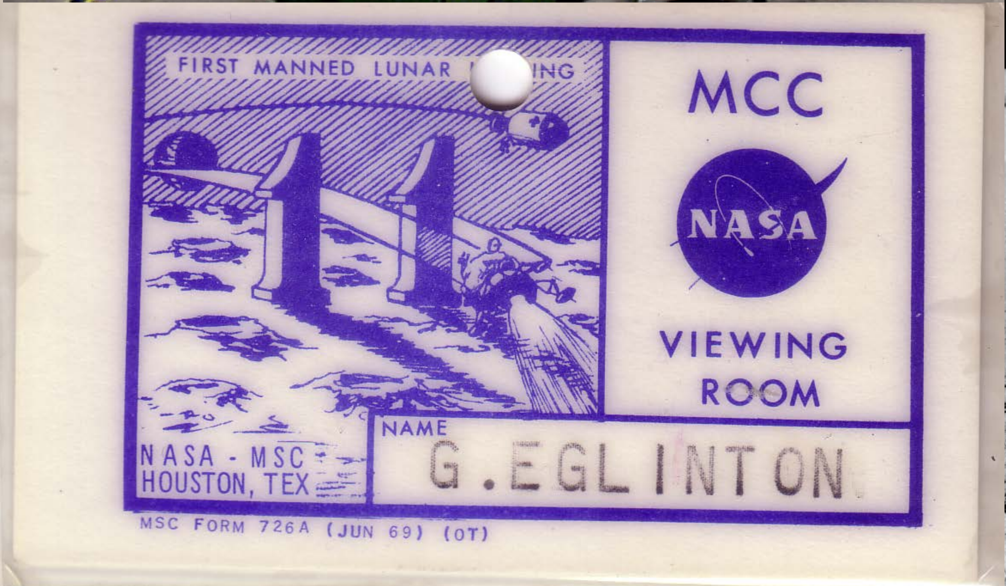
NASA-MSC
Houston, Texas

FIRST MANNED LUNAR LANDING

NAME

G
EGLINTON

SC FORM 1403 (JUN 69) (OT)



FIRST MANNED LUNAR [illegible]ING

MCC

NASA

VIEWING
ROOM

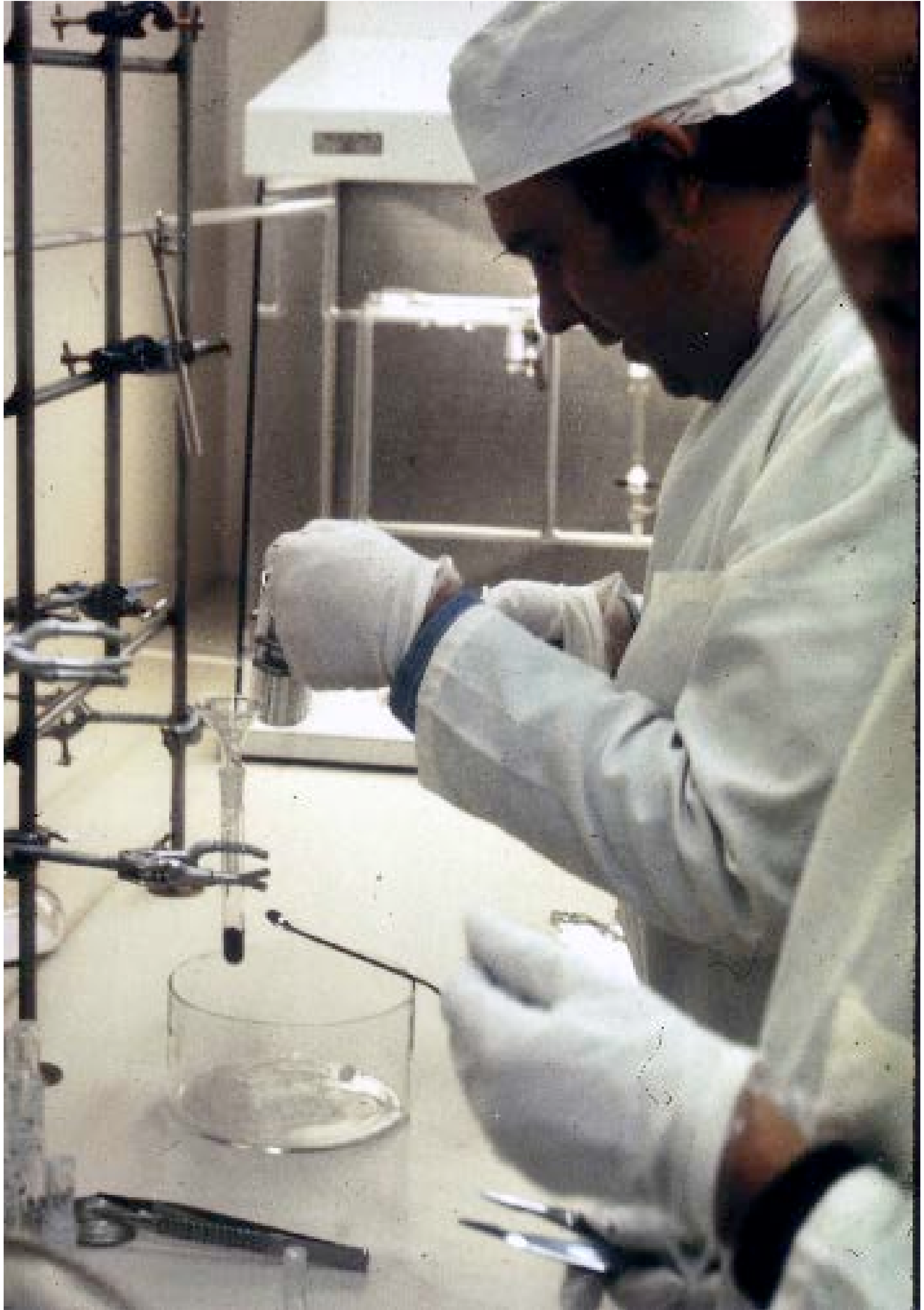
NASA - MSC
HOUSTON, TEX

NAME G.EGLINTON

MSC FORM 726A (JUN 69) (OT)


SCI CAM I
CAMERA
10063,0 R6P
10064
S5
10064
R1F
10065
S5
10065 RIP
10066,0
S4P
10067,0
R1P
10062,0
R3P
10068,0
S5P
10069,0
S4P

1969: Lunar Sample Analysis Planning Team (LSAPT) in session!

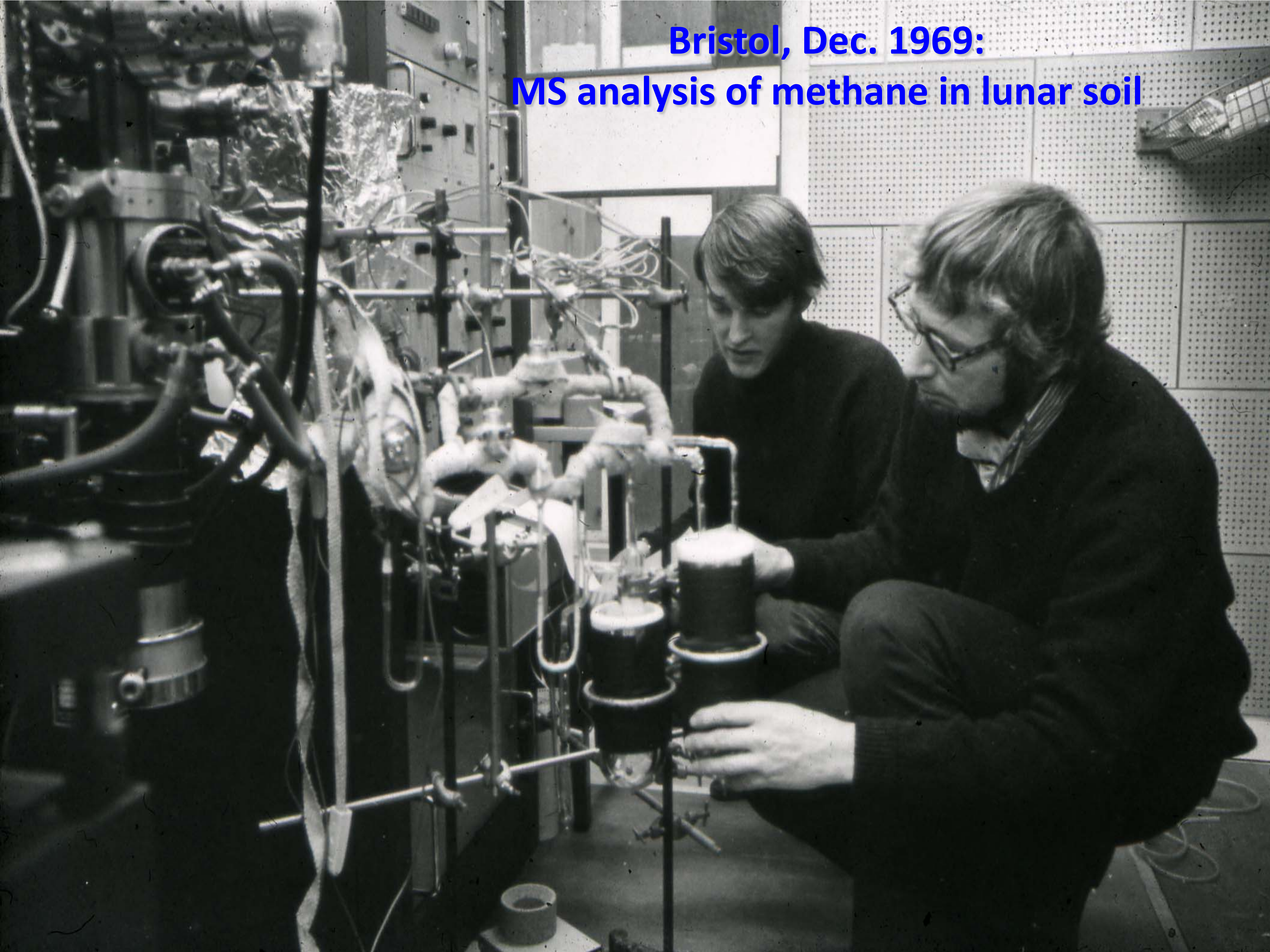


Bristol: September 1969:
Lunar sample comes to Bristol. James Maxwell and Colin Pillinger transfer moon dust from the NASA container-with some spillage!

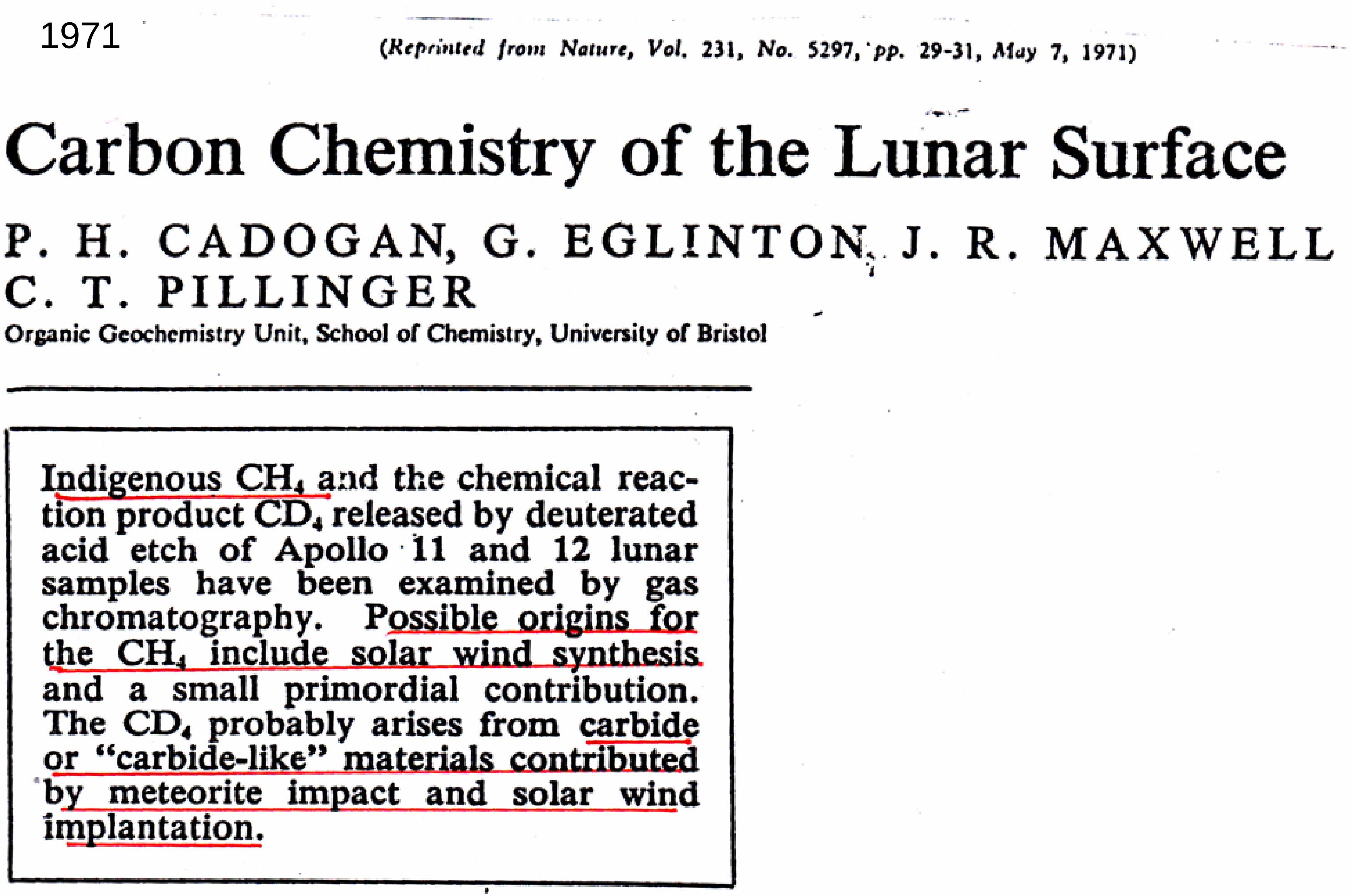
Noel 1969: So what did we find in Apollo 11 soil ?

- As expected - no biomarker alkanes above laboratory background (1 part in 10^9). Therefore , no life — surprise, surprise!!
- But we did find something else we were looking for
  - Methane generated in the lunar soil by solar wind bombardment of carbon and hydrogen!

Bristol, Dec. 1969:
MS analysis of methane in lunar soil



1971



(Reprinted from Nature, Vol. 231, No. 5297, pp. 29-31, May 7, 1971)

Carbon Chemistry of the Lunar Surface

P. H. CADOGAN, G. EGLINTON, J. R. MAXWELL
C. T. PILLINGER
Organic Geochemistry Unit, School of Chemistry, University of Bristol

Indigenous CH_4 and the chemical reaction product CD_4 released by deuterated acid etch of Apollo 11 and 12 lunar samples have been examined by gas chromatography. Possible origins for the CH_4 include solar wind synthesis and a small primordial contribution. The CD_4 probably arises from carbide or "carbide-like" materials contributed by meteorite impact and solar wind implantation.

1970s on: The rest of OGU was busy discovering the joys of GC-MS.

- What were all these compounds they were finding?
- Where had they come from?
- Which organisms had made them?

    - Potential Biomarkers???

- Why did some compounds found in geological samples have such changed structures and stereochemistries?

    - Maturity Indices had arrived.

- By the 70's and 80's we were into the new era *of on-line computer acquisition and processing* .

- The game of magic number chasing , e.g. *m/z* 85, 191, 217… often had instant and dramatic rewards:

Find one compound and get a whole homologous series free!

Ph.D. theses and publications flowed in torrents!

Playing with mass number plots was more fun for some graduate students than early computer games and you could get a Ph.D. at the end of it!

Molecular proxies were "in".

=

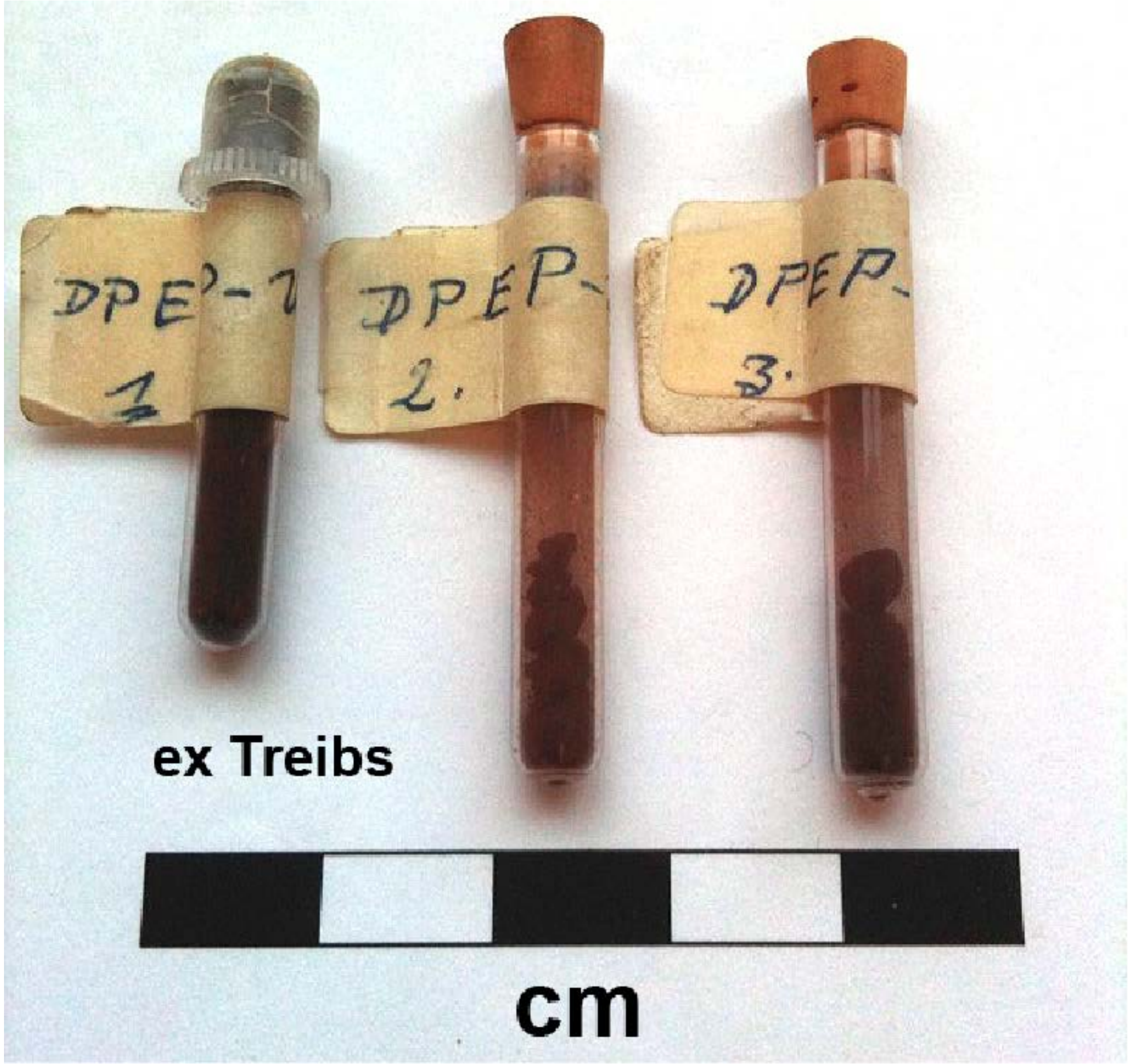
The 90’s and into the new millennium

- These two decades witnessed the full flowering of the computerised, coupled chromatographic and mass spectrometric technologies , with direct sampling of gases, solids, liquids and solutions. Even small proteins and polysaccharides could be coaxed into the ion sources. Even kerogen became a little less intimidating!

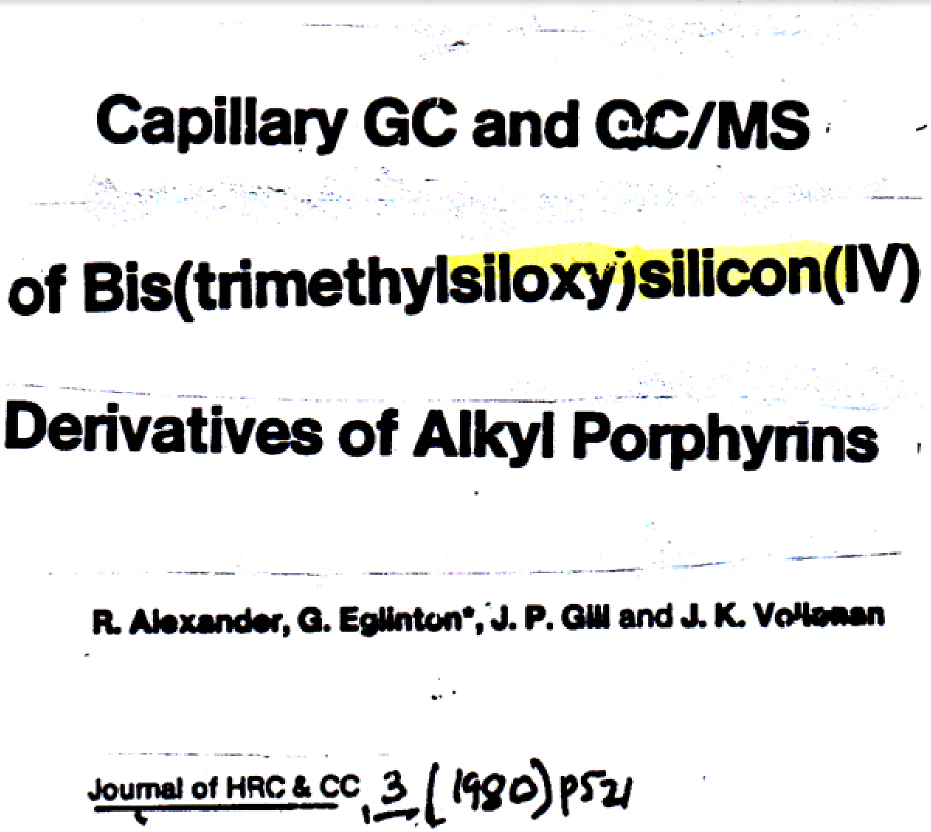
- However, these powerful, higher-resolution c-gcms techniques often revealed the paradigm-changing complexity of supposedlv pure compounds or simple mixtures.

- For example, Treibs’ 1938 ‘pure’ petroporphyrin samples were actually mixtures, though mainly the right compounds !

Treib's original DPEP samples given to James Maxwell and stored in the deep freezer at OGU at Bristol.



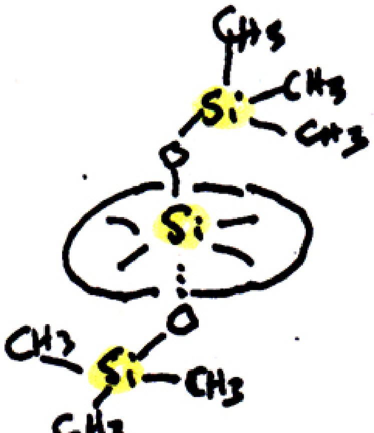
1980: Getting porphyrin rings to 'fly' in the GC!

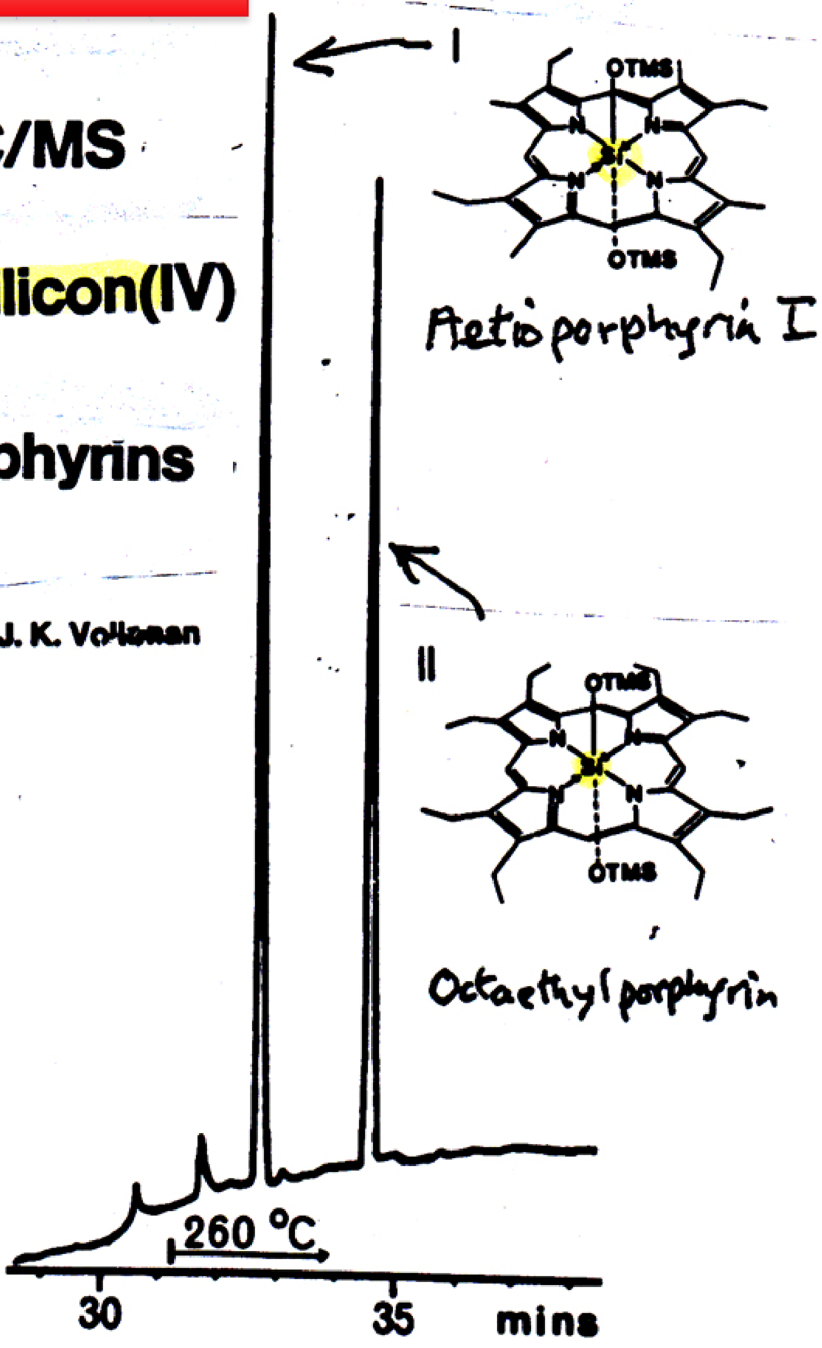


Capillary GC and GC/MS of Bis(trimethylsiloxy)silicon(IV) Derivatives of Alkyl Porphyrins

R. Alexander, G. Eglinton*, J. P. Gill and J. K. Volkman

Journal of HRC & CC, 3 (1980) p521





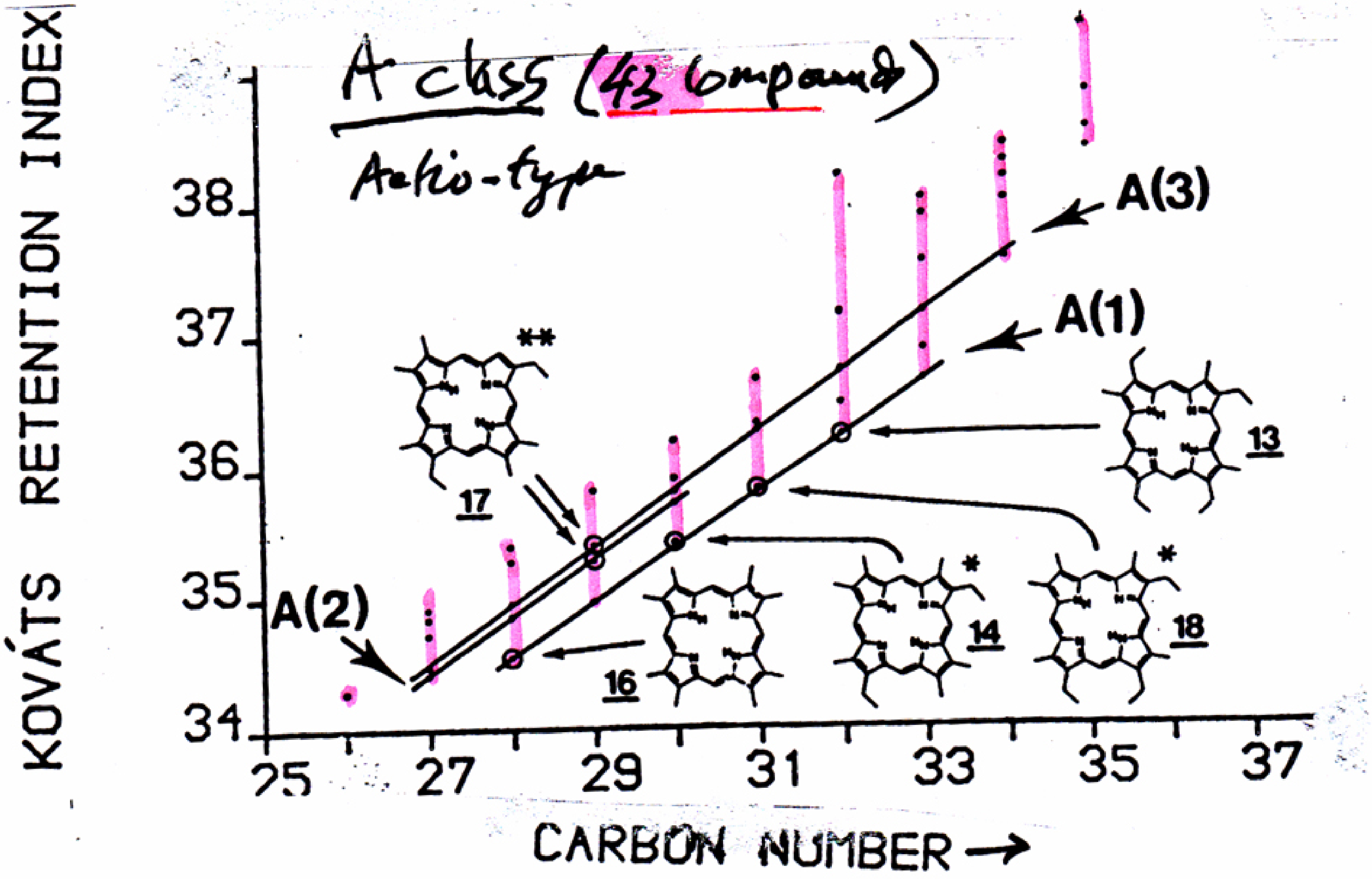
In 1985 we used a similar C-GC-MS technique with silicon IV derivatives to reveal that the porphyrin fraction isolated from the Green River Shale bitumen, Gilsonite , comprised at least 100 isomers and homologues!

COMPUTERISED CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC STUDIES OF THE PETROPORPHYRINS OF THE GILSONITE BITUMEN (EOCENE, U.S.A.)

(as bis TBDMSO Si IV)

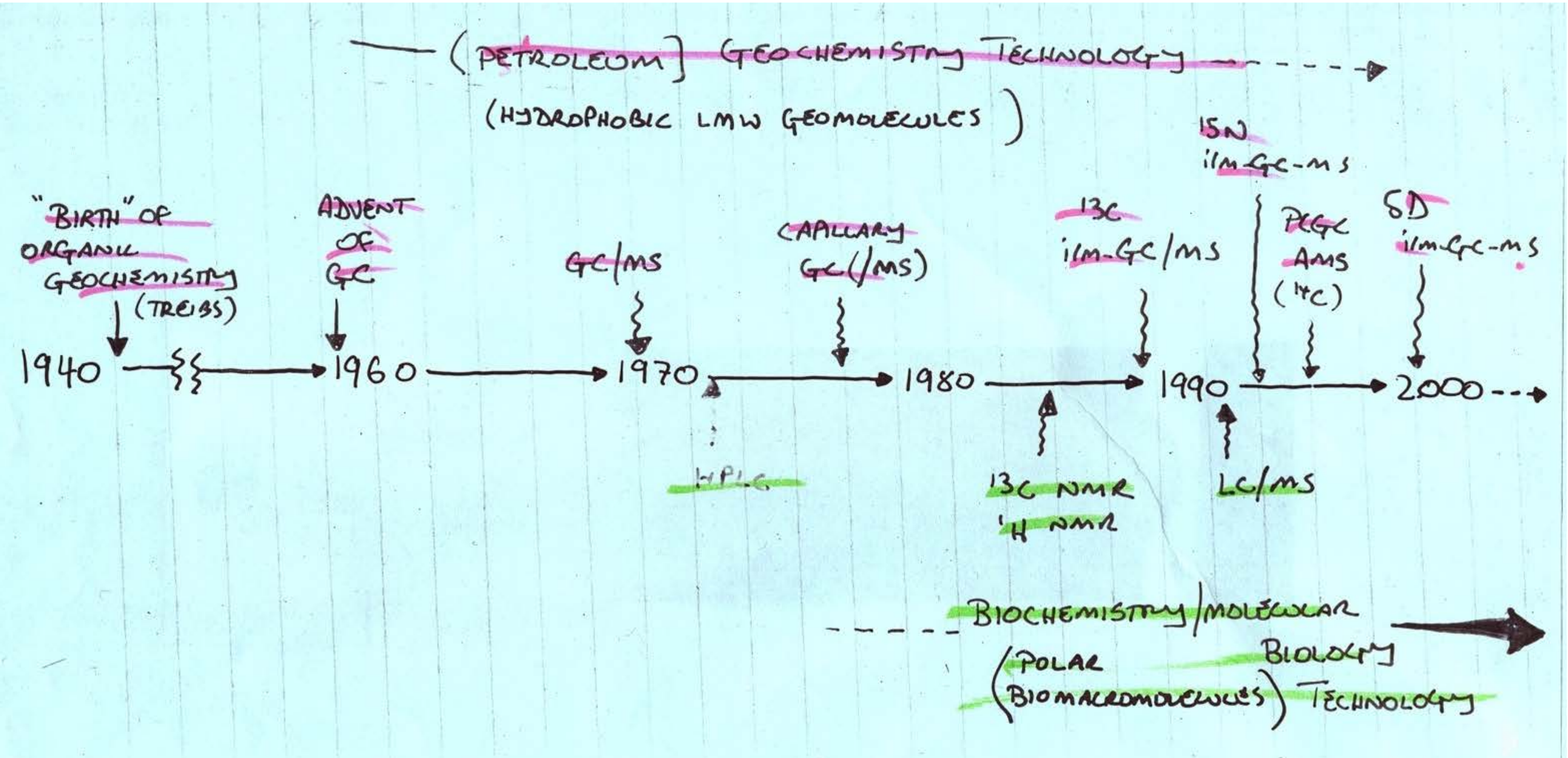
Journal of Chromatography, 350 (1985) 37–62

J. P. GILL, R. P. EVERSHED*, M. I. CHICARELLI, G. A. WOLFF, J. R. MAXWELL and G. EGLINTON*



In the late 1980s another giant leap forward came out of John Hayes' lab: compound-specific, on-line stable isotope GC-MS and LC-MS .

- A whole new window opened up into the origins and histories of molecules and the biogeochemical pathways of ecosystems.



EVOLUTION OF ANALYTICAL APPROACHES IN ORGANIC GEOCHEMISTRY

So, looking forwards from 1969, what specific problems did the OGU at Bristol tackle with our GC-MS instrumentation?

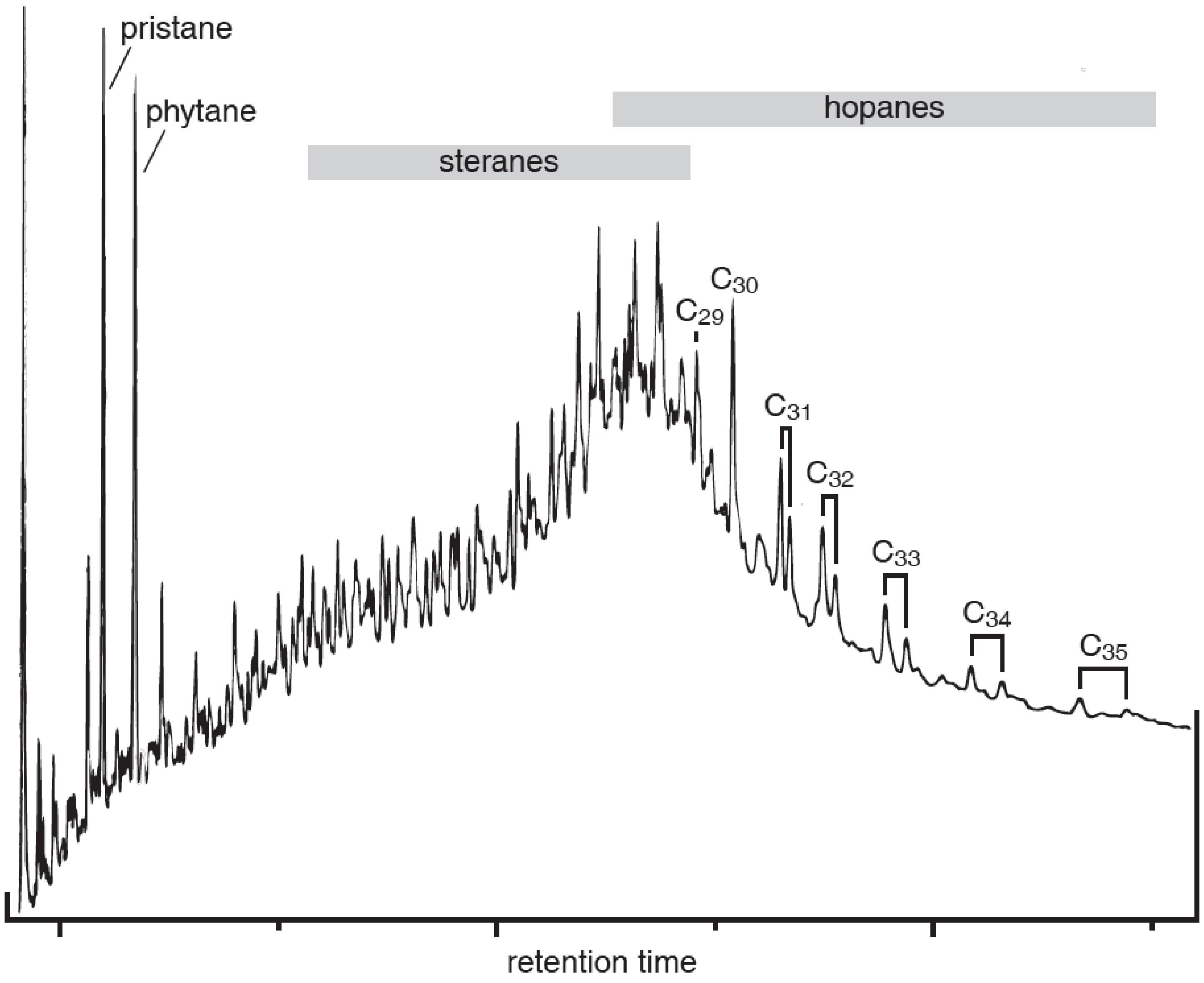
Example 1: the Paris Basin and petroleum

- This basin proved to be a great resource for biomarker studies of source rocks, and their diagenesis and maturation.
- But, sadly, no major oil reserve!
- Collaborators: IFP and Strasbourg.
- Funding: NERC, EEC and BP.

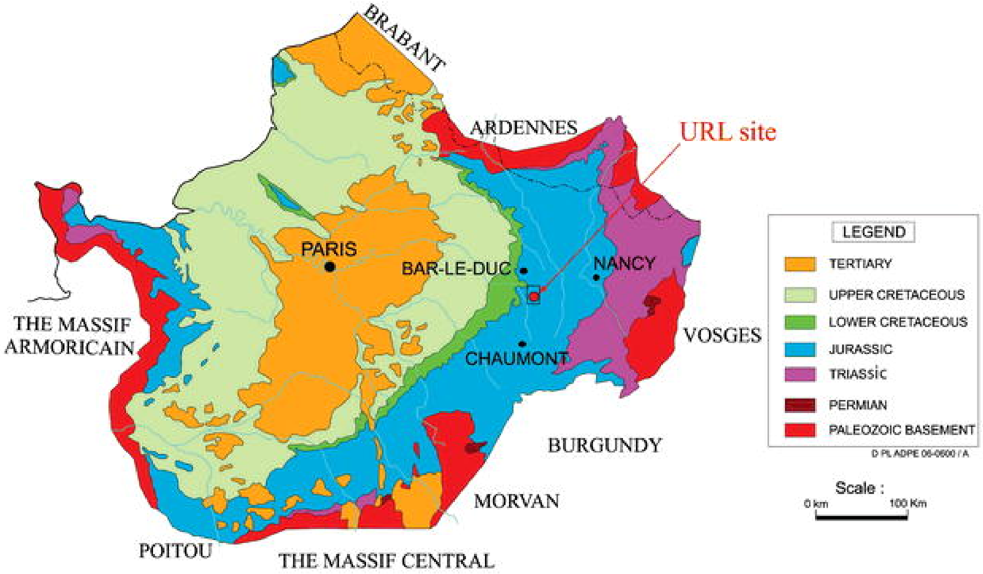
ALORS! – VOICI!- SOURCE ROCK BIOMARKERS, MOLECULAR MATURITY INDICES AND THE HOPANE STORY.

Gas chromatogram showing extended hopanes, 1975

deeply buried early Jurassic shale, Paris Basin



Paris Basin studies:



1974 Joint paper from Strasbourg and Bristol summarizing the hopane biomarker story: the mysterious and ubiquitous doublet peaks are explained!

And, in 1976 a major biological source molecule of these geological ‘orphans’ was proven in *Acetobacter.*

By 1984 the hopane biomarkers become routine tools for assessing maturity in oil exploration.

Degraded and extended hopane derivatives (C_{27} to C_{35}) as ubiquitous geochemical markers

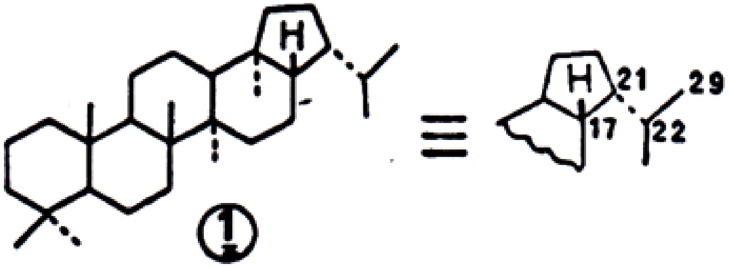
A. Van Dorsselaer, A. Ensminger, C. Spyckerelle, M. Dastillung,
O. Sieskind, P. Arpino, P. Albrecht and G. Ourisson

and

P.W. Brooks, S.J.Gaskell, B.J. Kimble, R.P. Philp, J.R. Maxwell
and G. Eglinton

Table 1. Hopane derivatives from geological sources*.

Tetrahedron Letters
No. 14, pp 1349 - 1352, 1974.



EARLY 1980's:
Example 2: Biomarkers in Extreme Environments

YELLOWSTONE HOT SPRINGS

- Various simplified ecosystems with water to boiling point, extreme acidity etc.,. Stratified microbial mats grow, but with very limited species tolerance.

- WHY? Excellent site for exploring biolipids vs microbial species and process relationships in the mat layers.

- David Ward, expert microbial ecologist with National park clearance, in residence at University in Bozeman, Montana.

AND what a place! Bears too.

Comment: Biomarkers & Microbiology

- Microbial world now seen as the dominant component of our biosphere and key to the major biogeochemical cycles.
- The microbial world generally lacks preservation potential in terms of morphology and hence, its fossil record is extremely incomplete.
- *The molecular record of microbial activity* is thus **a major resource**, especially when combined with the isotopic records. Source biomolecules include:
    - Mitochondrial DNA (only 17,000 base pairs!)
    - Chlorophyll , carotenoids, quinones and other pigments
    - Glycerides and polar lipids, including branched and cyclic ethers, hopanoids and sterols as membrane components.

Cross section microbial mat from Octopus Spring

Yellowstone Hotsprings

Example : Extremophile Microorganisms -

The Hyperthermophiles. Hyperthermophile bacteria and archaea live in hot springs (up to 110° C), as in Yellowstone National Park, USA. Their enzymes are termed **'thermozymes'.**

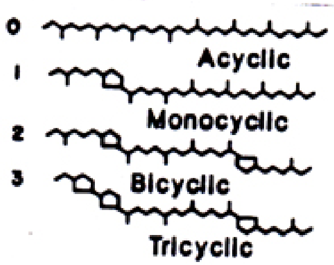
Archaebacterial lipids in hot-spring microbial mats

from Nature, Vol. 318, No. 6047, pp. 656-659, 19 December 1985

D. M. Ward*, S. C. Brassell† & G. Eglinton†

* Department of Microbiology, Montana State University, Bozeman,
Montana 59717, USA
† Organic Geochemistry Unit, University of Bristol,
School of Chemistry, Cantock's Close, Bristol BS8 1TS, UK

Modern and ancient sediments contain a multiplicity of organic compounds which record a partial history of the organisms that contributed them[1]. Interpretation of this record depends on the knowledge of links between the molecular markers and their biological, notably microbial, sources. This goal is made difficult by the complexity of modern sediment communities and the general lack of studies which directly tackle the relationships between the microbial inhabitants of natural systems and their associated organic compounds. We now report the first direct comparison between isopranyl ether-linked lipids in hot-spring microbial mats and the observed populations of archaebacteria. The particular mats examined are from Yellowstone National Park and may represent modern analogues of Precambrian communities preserved as stromatolites[2,3]. As relatively simple microbial systems, they are suitable for validating specific molecular markers as indictors of microbial populations within a natural community. We find that the phytanyl and biphytanyl ethers reflect the measured distribution of methanogenic archaebacteria.



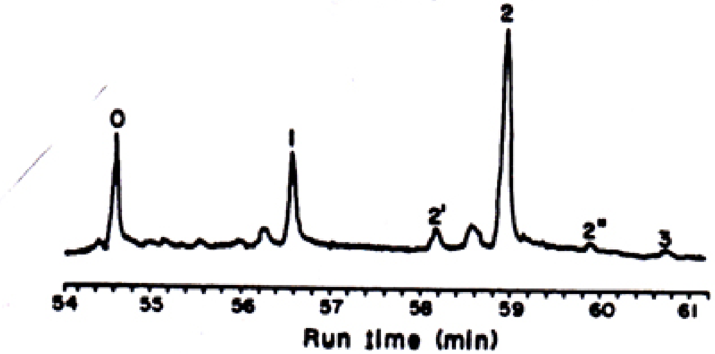


Fig. 1 Glass capillary gas chromatograms of alkanes derived from the ether cleavage procedure from: . Nymph Creek 47 °C mat,

1993:
Example 3: Biomarkers in Extreme Environments

WHOI-IFREMER EXPEDITION : “BLACK SMOKERS” AT THE BOTTOM OF THE ATLANTIC OCEAN

TRANSPORT: IFREMER’s Jean Charcot , WHOI’s Atlantis II, and the 3 man submarine “ALVIN”.

COLLABORATOR: Ecologist/submariner: Cindy Van Dover

WHY: Hydrothermal ocean floor vents are species - limited, chemosynthetic, non-photosynthetic life zones, ideal for biolipid/ecosystem studies.

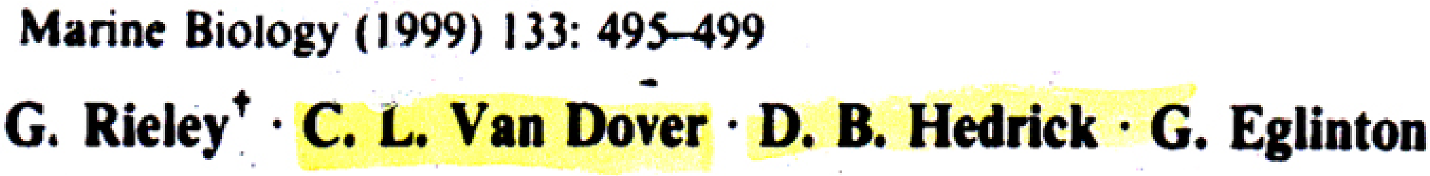
What biogeochemistry fuels the enormous swarms of blind shrimp feeding on the hot sulfide vents at the Mid-Atlantic Ridge, 3.6 Km down on the ocean floor?


NA
Iceland
Europe
Africa
South America
Menez Gwen
Saldanha
Lucky Strike
Rainbow
Atlantis F.Z.
Broken Spur
Lost City
TAG
Snake Pit
Logatchev
50°
40°
30°
20°
10°
0°
60° W
40°
20°
0°


NNESSEE.
VI
DS HOLE


Ifremer

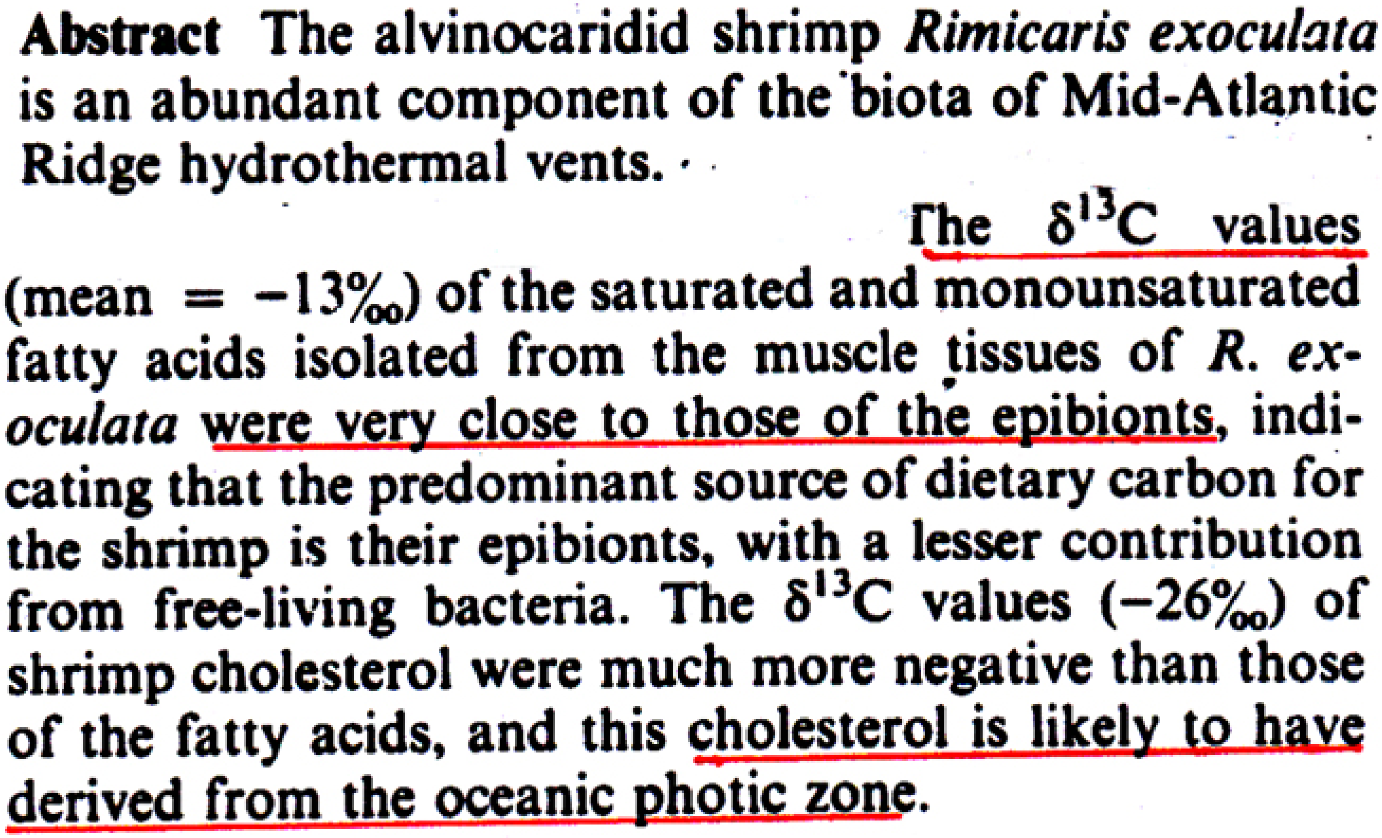

Baxter Healthcare Corporation
Scientific Products Division
ALVIN 2610
-DBH-5-16
Centimeters



Marine Biology (1999) 133: 495–499

G. Rieley† · C. L. Van Dover · D. B. Hedrick · G. Eglinton

Trophic ecology of *Rimicaris exoculata*: A combined lipid abundance/stable isotope approach



Abstract The alvinocaridid shrimp *Rimicaris exoculata* is an abundant component of the biota of Mid-Atlantic Ridge hydrothermal vents.

The $\delta^{13}C$ values (mean = -13‰) of the saturated and monounsaturated fatty acids isolated from the muscle tissues of *R. exoculata* were very close to those of the epibionts, indicating that the predominant source of dietary carbon for the shrimp is their epibionts, with a lesser contribution from free-living bacteria. The $\delta^{13}C$ values (-26‰) of shrimp cholesterol were much more negative than those of the fatty acids, and this cholesterol is likely to have derived from the oceanic photic zone.

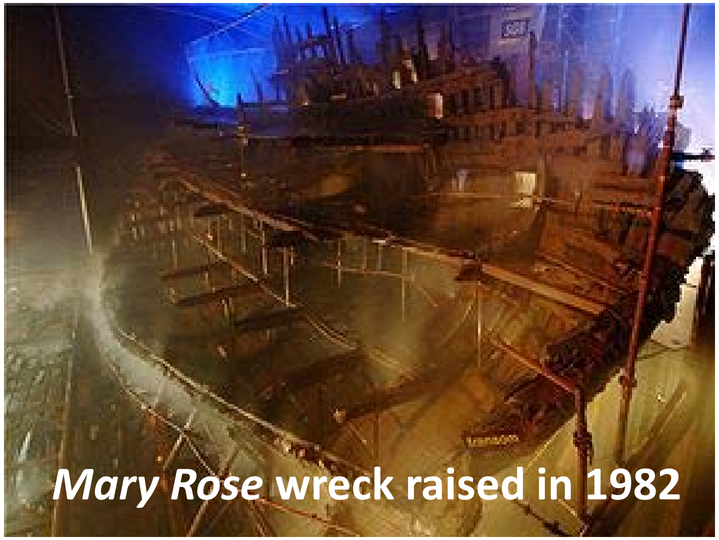
MID 1980's :
Example 4: Biomarkers and Archaeology

King Henry VIII's Flagship - the *Mary Rose* sank off Portsmouth, July 1545, sailing out to fight the French!

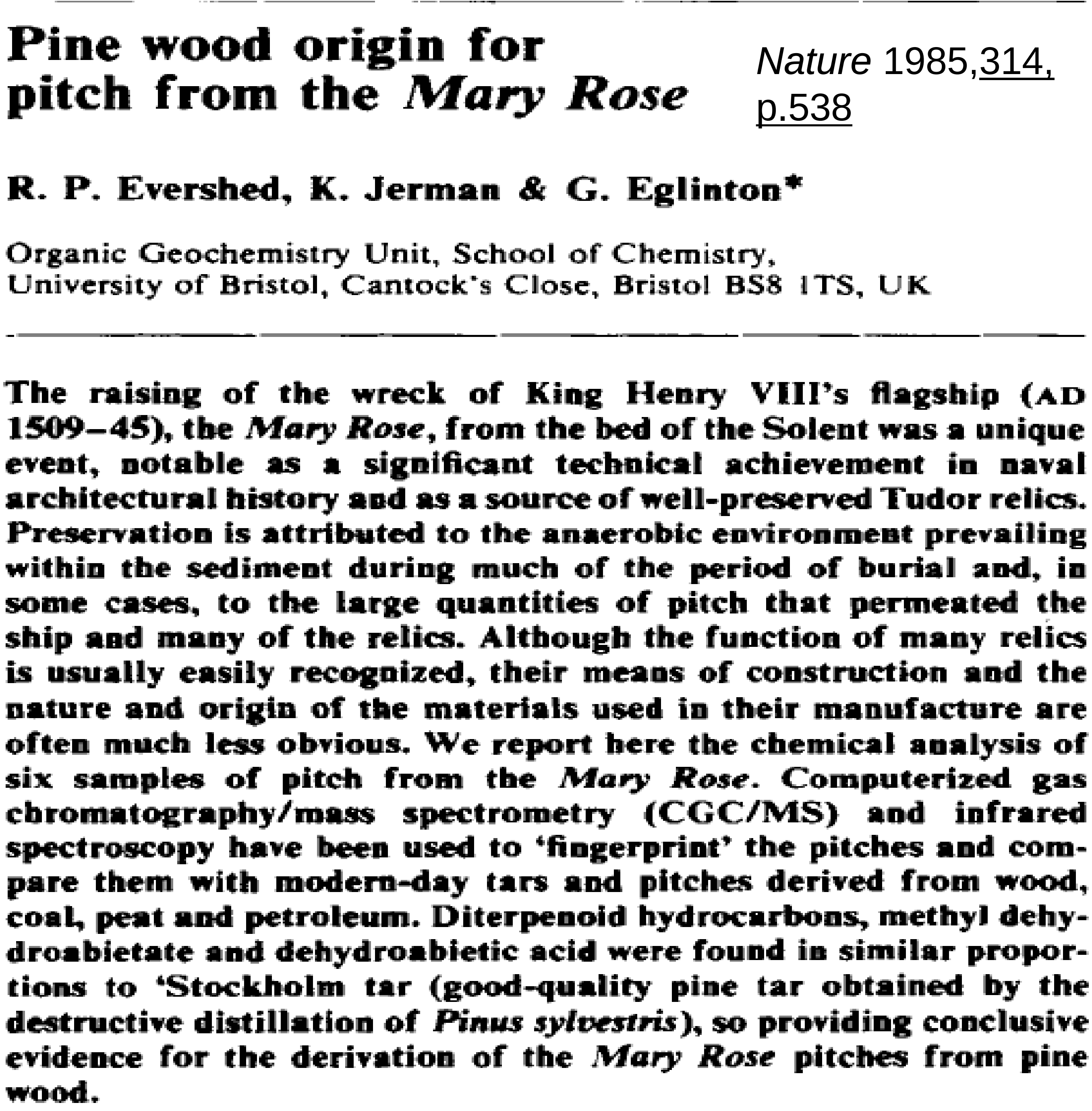
WHY: *What pitch/ tar did they use to make warships watertight in the early 1500s*?

A good excuse to visit the wreck!

AND explore GCMS analysis of lipid distributions as a tool in archaeology.



Mary Rose **wreck raised in 1982**



Pine wood origin for pitch from the *Mary Rose*

Nature 1985,314, p.538

R. P. Evershed, K. Jerman & G. Eglinton*

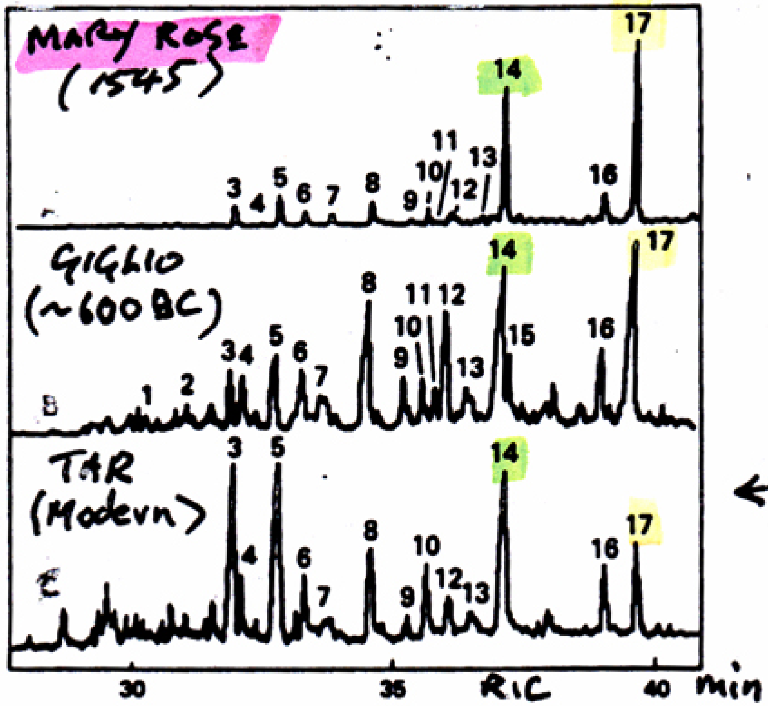
Organic Geochemistry Unit, School of Chemistry,
University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

The raising of the wreck of King Henry VIII's flagship (AD 1509–45), the *Mary Rose*, from the bed of the Solent was a unique event, notable as a significant technical achievement in naval architectural history and as a source of well-preserved Tudor relics. Preservation is attributed to the anaerobic environment prevailing within the sediment during much of the period of burial and, in some cases, to the large quantities of pitch that permeated the ship and many of the relics. Although the function of many relics is usually easily recognized, their means of construction and the nature and origin of the materials used in their manufacture are often much less obvious. We report here the chemical analysis of six samples of pitch from the *Mary Rose*. Computerized gas chromatography/mass spectrometry (CGC/MS) and infrared spectroscopy have been used to 'fingerprint' the pitches and compare them with modern-day tars and pitches derived from wood, coal, peat and petroleum. Diterpenoid hydrocarbons, methyl dehydroabietate and dehydroabietic acid were found in similar proportions to 'Stockholm tar (good-quality pine tar obtained by the destructive distillation of *Pinus sylvestris*), so providing conclusive evidence for the derivation of the *Mary Rose* pitches from pine wood.

ANALYST, MAY 1987, VOL. 112

Proof of a Pine Wood Origin for Pitch from Tudor (Mary Rose) and Etruscan Shipwrecks: Application of Analytical Organic Chemistry in Archaeology

Neil Robinson, Richard P. Evershed,* W. James Higgs, Katherine Jerman and Geoffrey Eglinton



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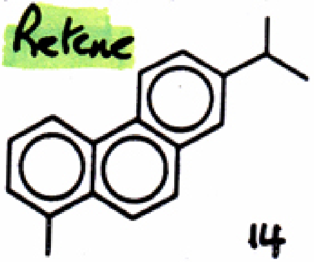
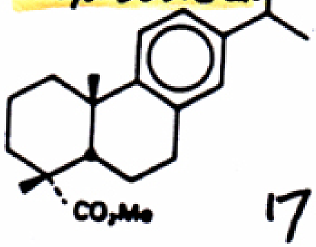


14

Dehydroabietate



17

Diterpenoids

← Modern 'Stockholm Tar' used to caulk ships.
(Destructive distillation of Pine wood)

Costa Concordia also sank at Giglio, 2600 years later, on January 13th 2012.

1990s:
Example 5: Pigments

Un chateau rose?
Oui!
Quincyte - A French fossil pigment

WHY STUDY?:

- *Organic pigments in rocks and fossils are rare, and fun to work on.*

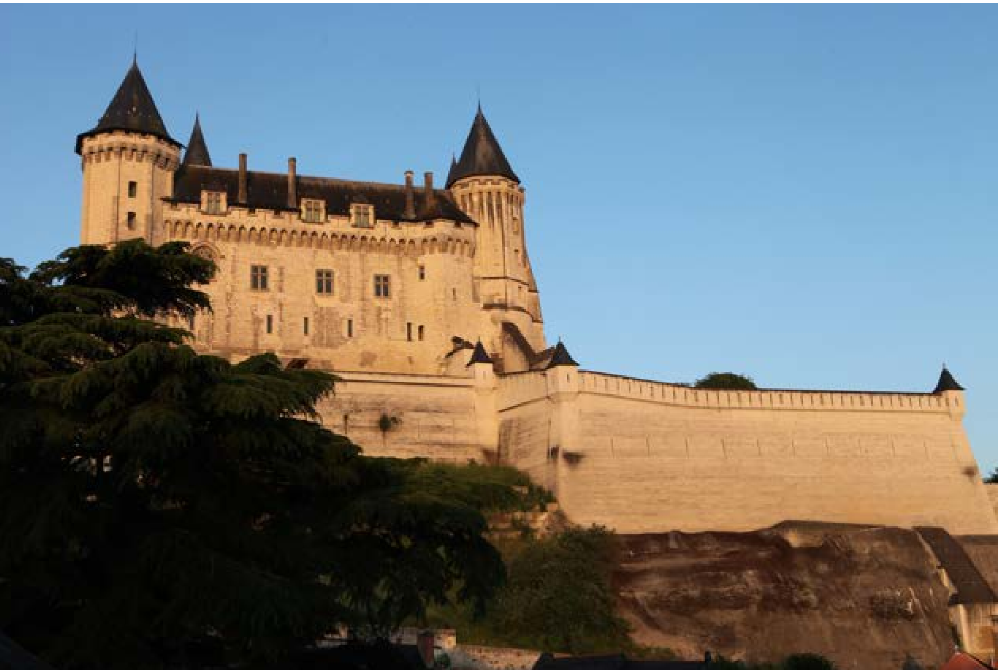
- The porphyrins are notable examples, and very recently, dinosaur melanin!

- Several families of fossil quinones (e.g., the Fringelites) are also known: was quincyte amongst them?.

Quincyte - A French fossil pigment

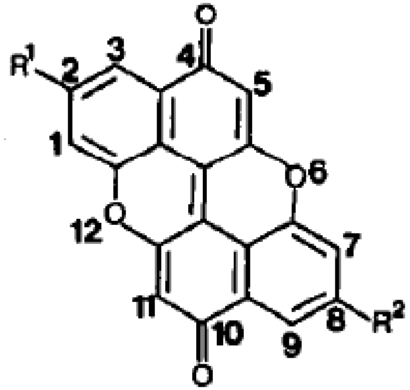
- In 1973, the 6th EAOG Meeting was held at Rueil-Malmaison and a field trip organised to sample the famous pink Eocene lacustrine limestone at a quarry near Quincy-sur-Cher. I brought choice pieces back to Bristol.

- Also provided was a magnificent lunch, abundant Quincy wine and a wander through the vineyard (dimly remembered!).



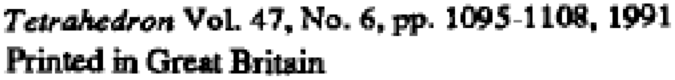

MEUNIER-LAPHA
Quincy
MEUNIER-LAPHA
PINOT ROS

In 1991 James Maxwell and colleagues published the full structure and synthesis of this elusive pigment , which defied extraction from the clay matrix (sepiolite) until liberated by dissolution of the rock in HCl , followed by HF.



R^1 = i-Pr
R^2 = i-Pr

2,8-di-isopropyl-peri-xanthenoxanthene-4, 10-quinone


Tetrahedron Vol. 47, No. 6, pp. 1095-1108, 1991
Printed in Great Britain

- **Quincyte** is possibly of fungal origin, like several other quinone pigments.

- Biosynthesis of this C26 quinone and other less abundant fossil pigments of this type awaits study. 13C content might help?

- **Why are they preserved** ? The Maxwell team suggested may result from being trapped in the molecular sieve structure (5.6 x 11.0 Angstrom channels) of the clay mineral (sepiolite) matrix.

- Would X ray studies reveal this?

1975 onwards:
Example 6: Molecular Proxy Records in Seafloor Sediments

- International Drilling Projects (DSDP, ODP) from 1968 used large drilling ships to obtain long cores of sediments from ocean floors.
- On board international teams sampled, characterised and dated sediment layers and engaged in interdisciplinary collaboration.
- Multiple samples, layer by layer, afforded lipid analyses (**molecular stratigraphy**) reflecting palaeoclimate/ depositional marine environments, terrigenous inputs and diagenetic/maturational changes in the sedimentary column.
- Bristol OGU worked on ODP Legs 108 (East tropical Atlantic) and 112 (Peru margin).

Devising a marine biomarker proxy.

The Logic:

- FIRST FIND C37 ALKENONES,

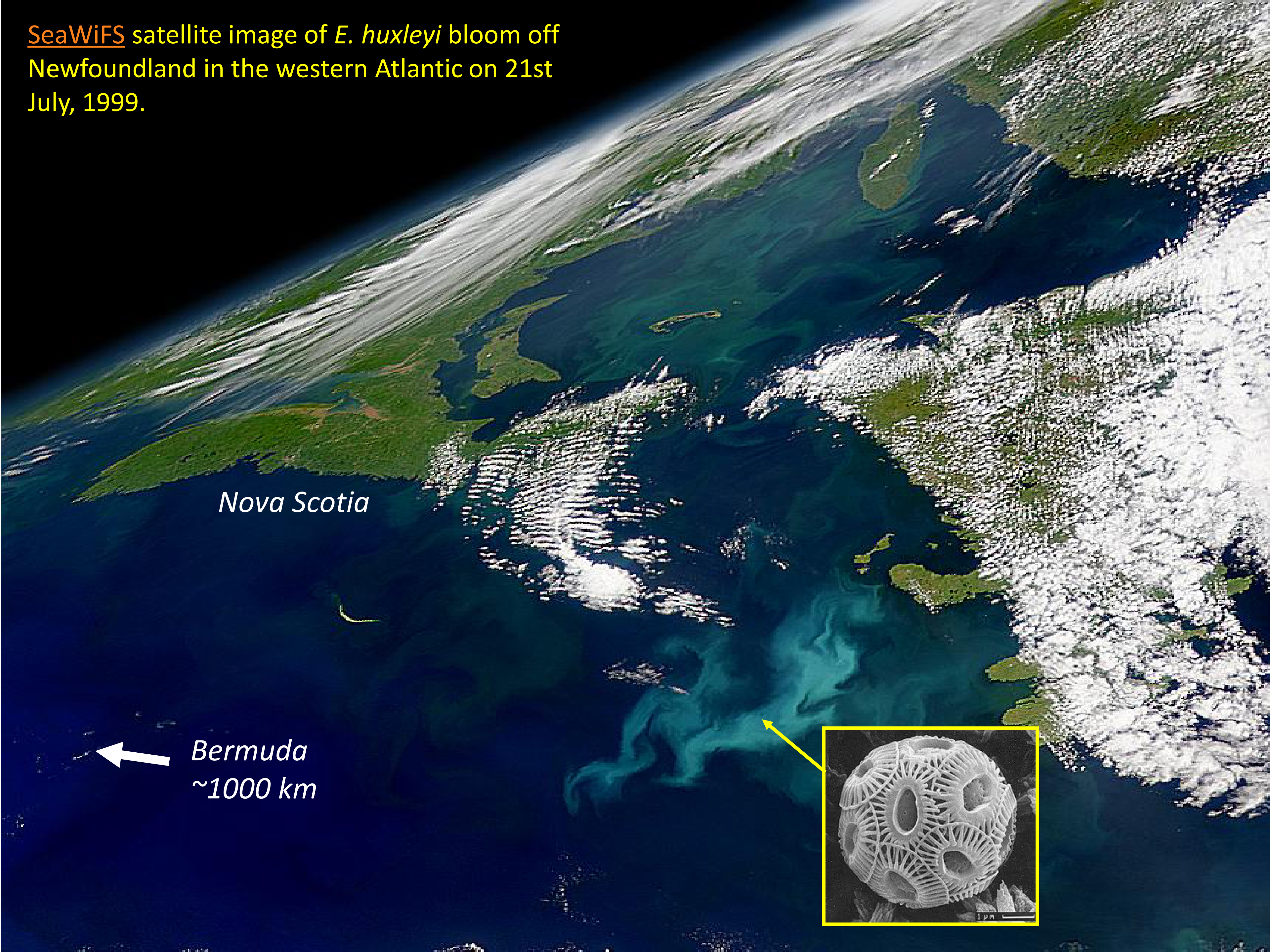
- THEN DETERMINE THEIR STRUCTURES and BIOSYNTHETIC ORIGIN .

- ESTABLISH THEIR FUNCTION.

- FINALLY, USE THEM TO FORMULATE AN SST PROXY - UK_{37}.

BUT DID IT HAPPEN LIKE THAT?

SeaWiFS satellite image of *E. huxleyi* bloom off Newfoundland in the western Atlantic on 21st July, 1999.



The Alkenones

Discovery, Inventory and Characterization

- **1976-7**: Novel, alkenone- type lipids found in the marine alga, *E. huxleyi* and in North Sea sediments (Sargent, Aberdeen, and Corner, Plymouth) .
- **1977-8**: Alkenones separated and partially characterized (Volkman, Bristol)
- **1978**: Alkenones reported and characterized in S. Atlantic sediments (de Leeuw et al., Delft).
- **1978:** Brassell (Bristol) finds alkenones in sediments from Japan Trench and other DSDP sites.

The Alkenones

Algae and water temperatures

- **1980:** Alkenone distributions found to differ in sediments from beneath cold *versus* warm water ocean sites.

- Brassell proposes that *E. hux.* algae make different alkenone distributions in cold and warm waters.

- Hence, we may have a **geo-thermometer**!

(*"a cusp of creation" or "a tragic self-delusion?", Tom Stoppard,Tel Aviv, May 19, 2008*)

- U^{K}_{37} proxy for paleo SST formulated:

$$U^{k}_{37} = 37{:}2/(37{:}2 + 37{:}3)$$

The Alkenones

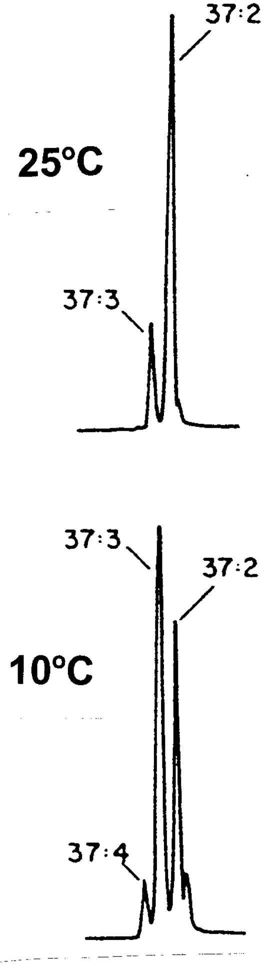
Laboratory calibration of U^K_{37} *vs* SST

- 1980-2. *E. huxleyi* cultures give **first plots of U^K_{37} *vs* temperature**.

Corner (Plymouth) and

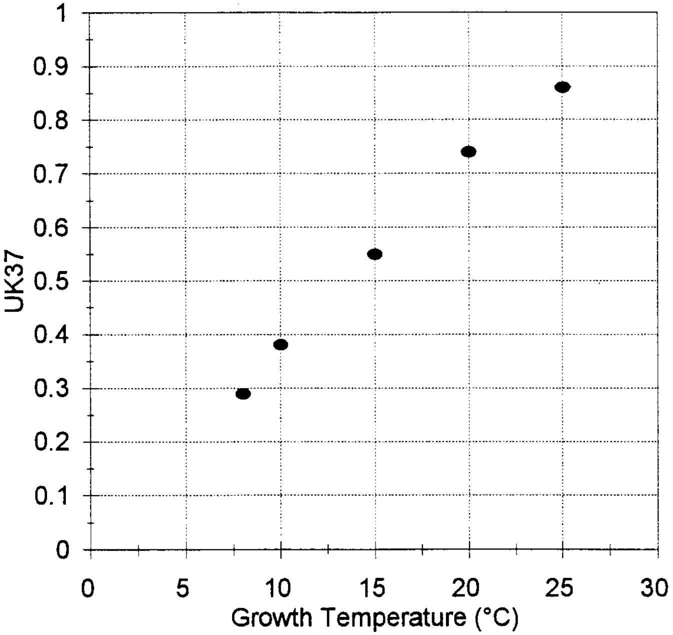
Brassell and Marlowe (Bristol).

After Marlowe, I. T. 1984. *Lipids as Paleoclimatic Indicators*. Ph.D. thesis, University of Bristol



Laboratory cultures

U^k_{37} = 37:2/(37:2 + 37:3)



The Alkenones

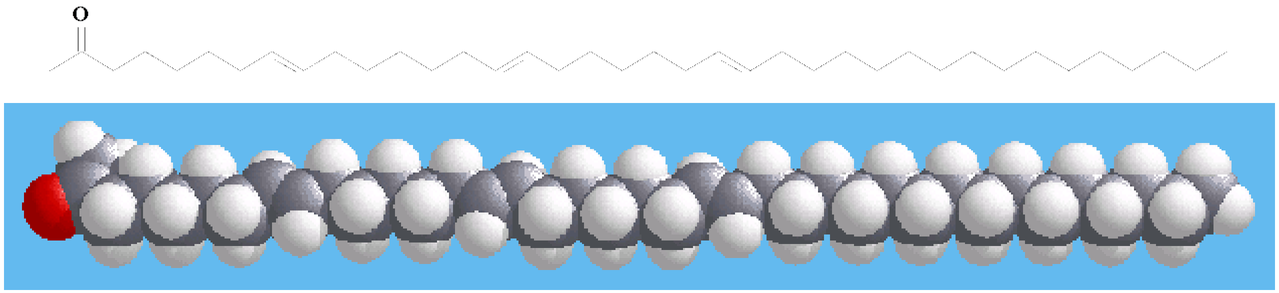
Validation and environmental calibration of U^K_{37} *vs* SST

- 1982-6. U^K_{37} data for worldwide marine bottom sediment samples plot as broad 'chevron' against latitude (i.e. sea surface temperature), peaking at Equator. Marlowe and Brassell (Bristol)

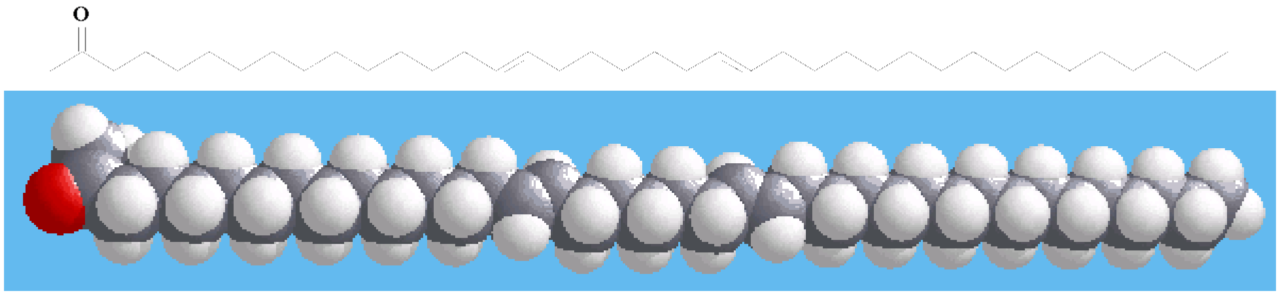
- 1984-6. When algae fed to copepods and fish in laboratory ecosystems, U^K_{37} unchanged in resultant fecal pellets. Corner (Plymouth), and Prahl (Bristol)

Alkenones

A few kinks and one oxygen atom – an algal biomarker for past sea surface temperature!



37:3



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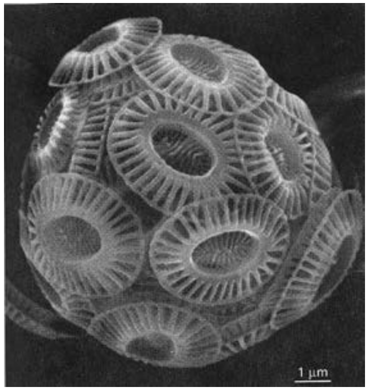


E. huxleyi

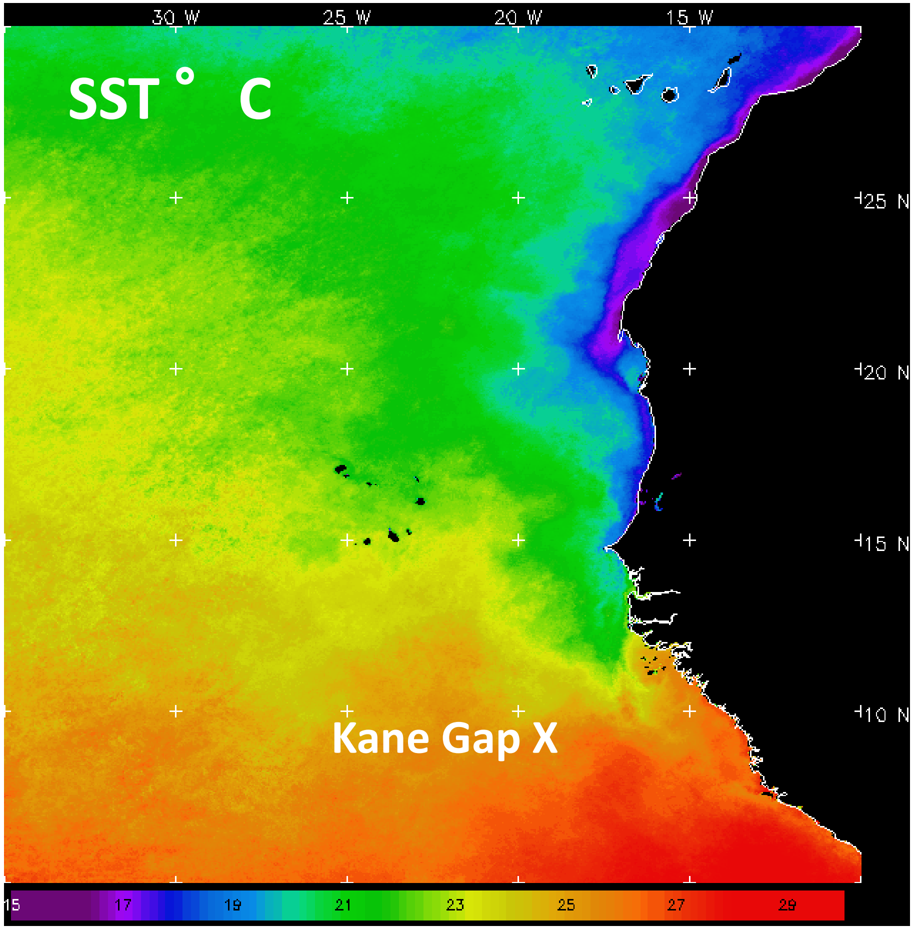
Using U^{K}_{37} to estimate paleo-oceanic SST: does it work?

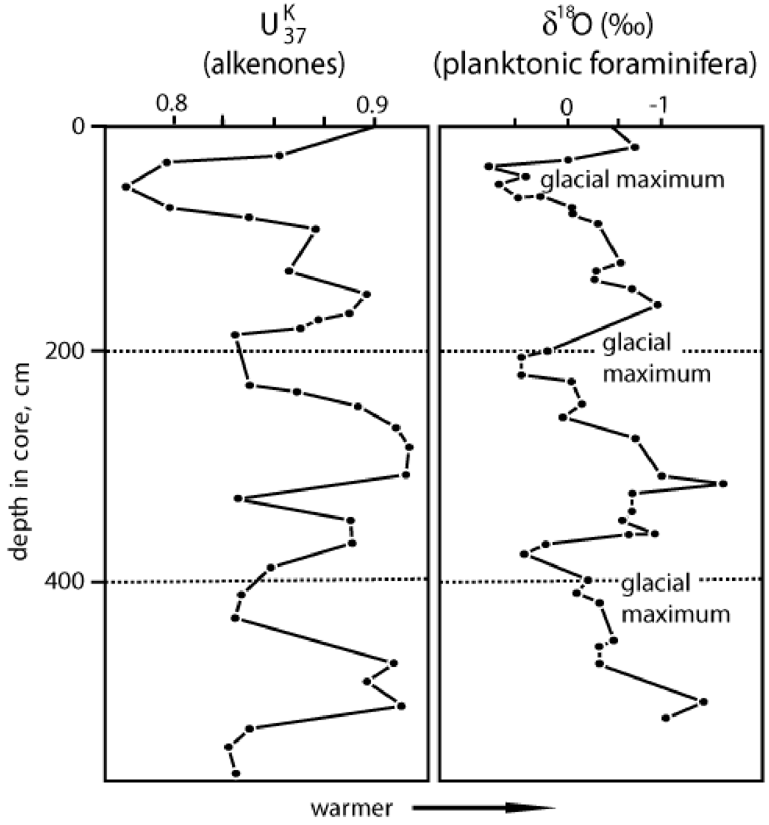
1984-6. A first test: Kane Gap, off NW Africa

Core chosen because oxygen isotope record downcore shows almost complete sequence of glacial/interglacial cycles over last million years. Also, sediments contain sufficient alkenones for analysis.

Brassell, Eglinton and Marlowe (Bristol)
and
Pflaumann and Sarnthein (marine geologists at Kiel).

First sediment depth plot of U^{K}_{37} parallels $\delta^{18}O$ (‰) of foraminiferal calcite (measure of SST and global ice volume). A Bristol-Kiel collaboration (12th EAOG, Juelich 1985 and Nature 1986, 320, 129-133). IT WORKS!




Alkenone unsaturation (U^{K}_{37}) and oxygen isotope ($\delta^{18}O$) stratigraphy

Kane Gap core, east equatorial Atlantic Ocean, 1984

Using U^{K}_{37} to explore oceanic paleo SSTs

- **1987. U^{K}_{37} vs SST field calibration** established using marine sediment trap and particulate samples.

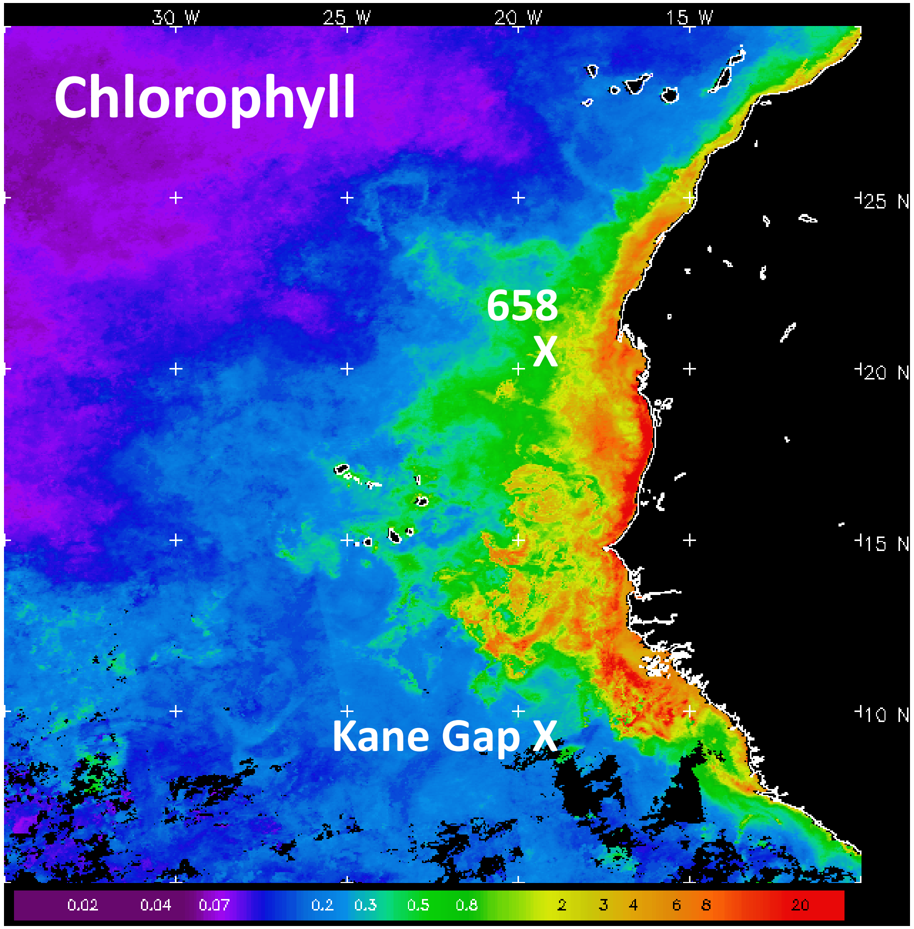
Prahl and Wakeham (WHOI, Mass.)

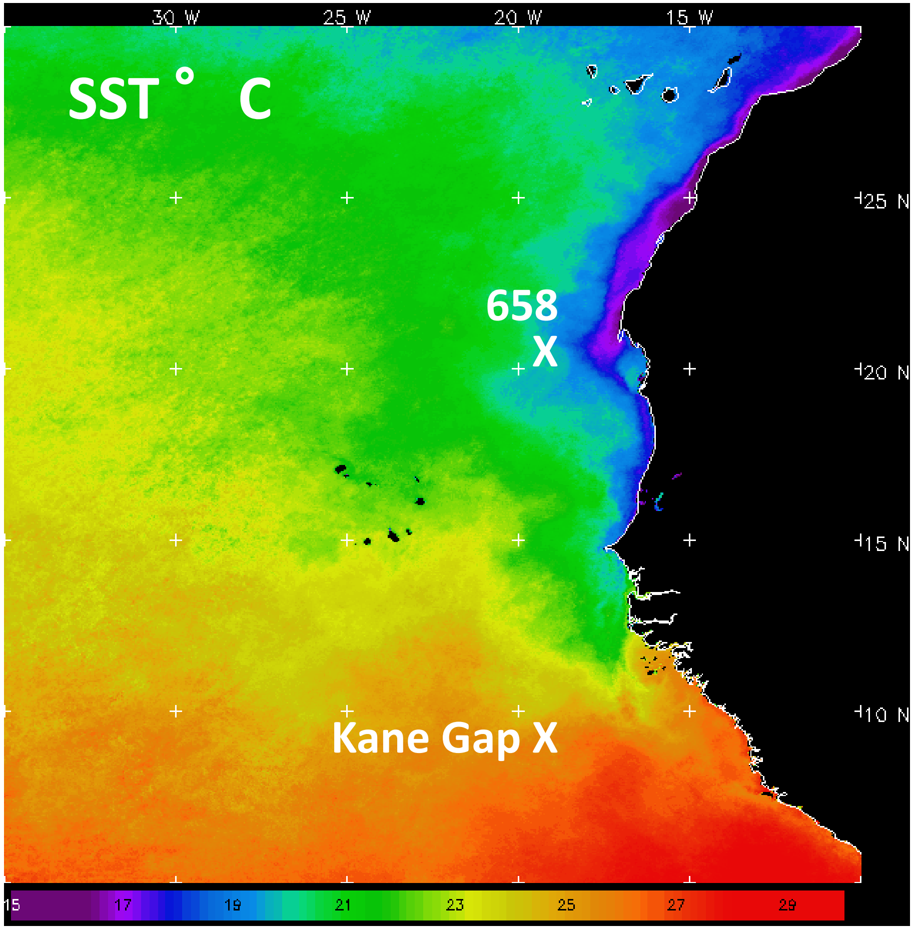
- **1991: First detailed test of U^{K}_{37}: ODP site 658, off NW Africa**

Site is in an upwelling area with high productivity and rapid sedimentation due to strong input of Saharan dust. Hence, **alkenone stratigraphy** could be examined at high time resolution by sampling at intervals a few cm apart downcore.

Eglinton, Bradshaw, Rosell (Bristol)
And
Sarnthein, Pflaumann, Tiedemann (Kiel).

Satellite Imagery:ODP site 658



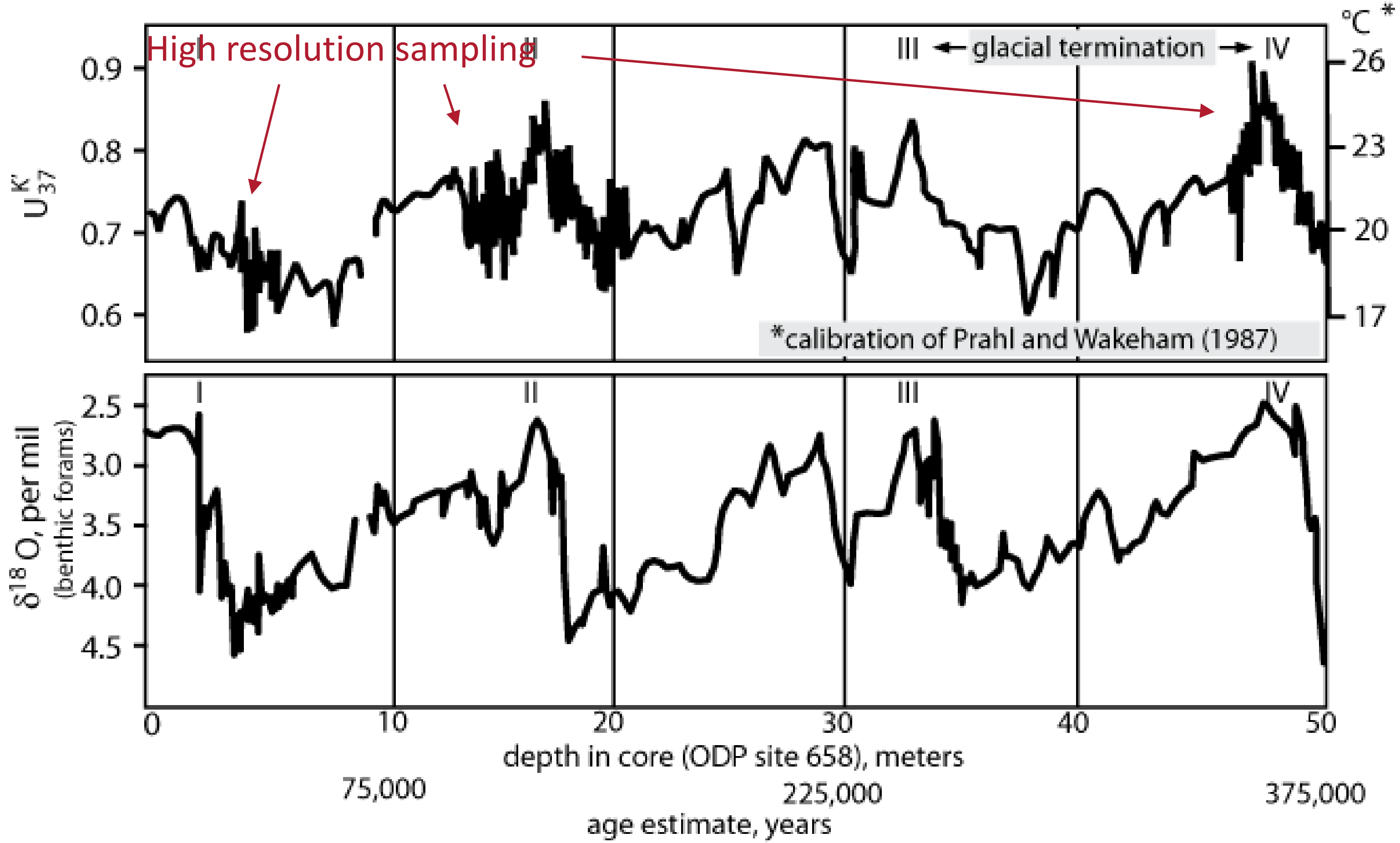


NW Africa, MODIS winter 2003

1991-
1992.

First alkenone U^{K}_{37} record of abrupt climate oscillations, 1991

Comparison with the foram ^{18}O record of 3 glacial cycles (tropical NE Atlantic)



Three high resolution sections of U^{K}_{37} SST stratigraphic records over last 375 kyr show **centennial scale oscillations of 2-3° C**. '*Were these abrupt SST oscillations linked to ocean circulation changes and meltwater events*'?

After Eglinton, et al. 1992. *Nature* 356:423–426

Alkenones: The Future?

Emiliania blooms wax and wane in the oceans down the centuries *but their **alkenones** persist as records of the temperature of the sea water in which they grew!*

- Alkenone data can be used to generate paleo SST records at high time resolution.
- Alkenones (*E. hux* etc) are virtually ubiquitous in measurable quantities in Late Quaternary marine sediments.

- *So, Global Paleo SST maps could be created using ocean-wide alkenone data-a massive effort, but possible with automated analyses.*

Global SST maps at single time horizons (e.g., the Last Glacial Maximum) would provide much-needed palaeo SST estimates for feeding into the General Circulation Models (GCMs) used to calculate past global climates.

AND FINALLY! BIOMARKERS AND MOLECULAR ORGANIC GEOCHEMISTRY: LOOKING BACK OVER THE YEARS.

Well, **what** did we do?

We have ‘visited’ the moon, been to the bottom of the Atlantic, sampled hot springs ,cored microbial mats, lake and ocean sediments and analyzed microorganisms, plants, animals, fossils, muds, bogs, rocks, pots, tars and petroleums.

We have been part of the successful communal effort showing that life **does** leave significant molecular records in the earth’s crust – even in the oldest sediments - and that **biomarker compounds** provide a wealth of **information about past biospheres and environments**.

The O.G. community now routinely uses **molecular indices and molecular proxies** to characterise present and past environments and to determine the operation of **diagenetic and maturational processes.**

2012: Where are we now?

- With compound-specific, stable isotope GC-MS and LC-MS and compound-specific 14C methodologies fully operational, new windows have opened up onto the origins and histories of molecules and the biogeochemical pathways within and between ecosystems.

- ***Stereochemistry, isotopes, the "omics", and microbial ecology and physiology are now all on board, so molecular organic geochemistry is getting really complicated --- and even more fun!***

But can we interlink biomarker systematics and the genetic and epigenetic revolutions to derive new understandings of evolutionary processes?

The Long Term Future?

- Using biomarkers to explore and understand the biosphere

- Coupling biomarker data with the geological record in search of a better **understanding of the evolution of life and life processes on this planet**

- **Solar system exploration?!**

(A tempting prospect : can biomarker methodology help establish whether or not Mars once harboured life ?)

FUTURE BIOMARKER RESEARCH WILL REQUIRE:

ACCESS TO NEW AND MORE POWERFUL ANALYTICAL INSTRUMENTS

ADEQUATE FUNDING FOR TECHNICAL SUPPORT

COLLABORATION WITH GROUPS EXPERT IN OTHER DISCIPLINES

PROJECTS WHICH INSPIRE BUT ARE BUILT ON A CORE EXPERTISE

LATERAL THINKING TO SPOT EMPTY RESEARCH NICHES

SYSTEMATIC 'STAMP COLLECTING' TO REVEAL THE 'ODD BALL'

AND, ABOVE ALL , CURIOSITY AS A DRIVER !

ALORS!   ENFIN LE FIN!   MERCI!