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Molecular recognition in allergic contact dermatitis to natural products

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Abstract- The chemical stuctures of natural skin allergens is discussed: the majority is electrophilic or "proelectrophilic" (i.e. they can be transformed in the skin into electrophiles. A number of natural and model skin allergens (or haptens), in particular optically active β-substituted-α-methylene-γ-butyrolactones such as tulipalins B, have been synthesized and their allergenic activity tested in the guinea pig. A remarkable enantiospecificity of the *in vivo* response is noted when the chiral center is close to the protein site of attack, the α-methylene group. Use of an *in vitro* model, reaction of selected haptens with a dipeptide, BOC-Cys (SH)-Ala-OMe, shows parallelism between the stereoselectivity of the Michael addition and enantiospecificity of the *in vivo* reaction, allowing some reasonable predictions to be made .

Allergic Contact Dermatitis (ACD) is a disease of the skin consecutive to the contact with some substances of natural or synthetic origin called allergens or haptens (ref. 1). The compounds responsible for this adverse skin reaction are generally electrophilic or "pro-electrophilic" and can react with nucleophilic groups in proteins. It is believed that haptens (or allergens) which are low molecular weight compounds penetrate into the skin, get bound to epidermal proteins and are taken up by the macrophage of the epidermis, the dendritic Langerhans cell (ref. 2) . The latter processes the hapten-protein adduct (called complete antigen) though pinocytosis and endocytosis and enzymatic hydrolysis leads to hapten-peptide conjugates which are reexpressed to the surface of the cell, along with elements of the major histocompatibility complex (MHC) called Ia or D_R antigen. The ternary hapten-peptide-MHC complex is presented to another cell which plays an important role, the T-lymphocyte , where it is recognized by receptors. A clonal selection and blastogenesis of those T-cells (helper cells) able to recognize the hapten then occurs in the node and the system is now ready ("hypersensitive") for another contact with the same or a related allergen. The whole process takes about 5-7 days. In the first stage (sensitization) nothing is visible on the skin and it is only if a second contact takes place. The process is then faster (12-24 hours) and results in skin inflammation (redness or erythema plus swelling or edema). Fortunately another subpopulation of T-cells also forms at the same time, suppressor cells, which are responsible for diminishing the inflammation reaction or even to suppress it. Not everybody will give an eczematous reaction when exposed to the same substance. The presence or absence of allergic contact dermatitis (ACD) is the result of a delicate balance between effector and suppressor T-cells. Hereditary factors seem to regulate this.

A molecular approach of the ACD problem addresses the following questions:

-What structural features make a compound an allergen (or a hapten)? (This should help in prevention of ACD).

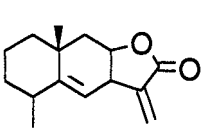
-How specific is the molecular recognition of a hapten?

-Is it possible to conceive compounds able to generate the formation of T-suppressor cells, in order to regulate this immunological phenomenon?

The following paper tries to give an answer to the first two questions.

NATURAL SKIN CONTACT ALLERGENS

There are thousands of substances responsible in nature for the development of ACD (ref. 3, 4) . Several families of plants are known for their agressivity on the skin. Thus, the Compositae family is one of the most offensive ones. The compounds responsible for ACD are sesquiterpene lactones such as, for instance alantolactone **1**. In India, a Compositae, inadvertently imported from the States in the 50's with wheat, grew to such an extent as to become a real national threat, this is *Parthenium hysterophorus* L. known as Congress Grass, particularly in the region of Poona. Another major problem, in Europe this time, is the benzoquinone, primine **2**, present in *Primula obconica* (Hance). Finally every American is aware of the adverse skin reaction to two species of Anacardiaceae plants, poison ivy (*Rhus radicans* L.= *Toxicodendron radicans* (L.) Kuntze, mostly in the East Coast) and poison oak (*Rhus diversiloba* Torr & Gray= *Toxicodendron diversilobum* Greene). Both contain urushiols, a mixture of 3-alkylcatechol derivatives, such as the pentadecycatechol **3** .



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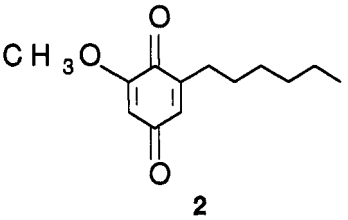

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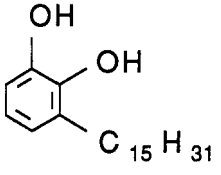

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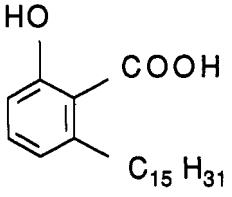
There are a number of other long chain phenols also responsible for allergic contact dermatitis. Beside examples taken from the Anacardiaceae family (such as cashew nut or *Anacardium occidentale* L., or mango or *Mangifera indica* L.) , one could mention salicylic acid derivatives present in ginkgo (*Ginkgo biloba* L.). We have studied recently Allergic Contact Dermatitis to ginkgo extracts in the guinea pig. Cross-reactions (i.e. a person allergic to a compound A can react to a compound B, structurally related) to ginkgo in poison ivy-sensitive patients have been described. It was believed that ginkgolic acid **4** could be metabolized into cardanol **5**, which could in turn be reoxidized into a catechol, present in *Rhus* plants.



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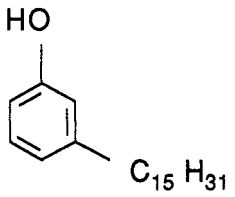

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In guinea pigs, however, no cross-reactions between ginkgo-sensitized animals and poison ivy-sensitized ones was observed (ref.5).

Famous other examples of skin contact allergens are α-methylene-γ-butyrolactone (also known as tulipalin A), present in tulips, quinones in tropical woods, aldehydes in cinnamon (cinnamaldehyde) or in perfumes (example, citral in lemongrass) etc.

MOLECULAR MECHANISMS OF ALLERGIC CONTACT DERMATITIS (ACD) INDUCTION

The few examples shown above show that the majority of natural skin haptens are electrophiles and in particular Michael acceptors. This is the case obviously for α-methylene-γ-butyrolactones, sesquiterpenic or not, quinones, α,β-conjugated aldehydes

such as cinnamaldehyde. Some are prohaptens, i.e. they must undergo a chemical transformation in order to become truly allergenic. Thus, catechols are known to be easily oxidized (either *in vivo* or *in vitro*) into reactive o-quinones. Aminoacid candidates for reaction with skin haptens are mostly cysteine with its SH group and lysine with its ε-NH_2 function.

By using a model skin allergen, ^{13} C-methyl alkylsulfonate:

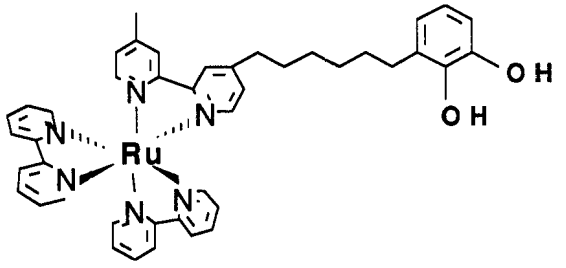
$$CH_3\text{-}(CH_2)_{11}\text{-}SO_3{}^{13}CH_3$$

and a model protein, human serum albumin, HSA, we have demonstrated by ^{13} C-NMR that lysines were involved in hapten-protein covalent bond formation. The only "free" cysteine present in HSA did not react, but there was some methionine-hapten bond formation (ref. 6).

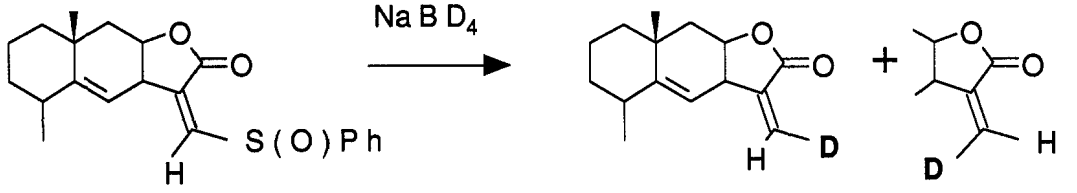
In order to try and establish some structure-activity relationships of the sensitizing (i.e. contact allergy inducing) substances, it seemed of interest: a) to label natural haptens in order to follow them in the skin or in skin extracts and b) to synthesize models of natural skin haptens.

LABELLING SKIN HAPTENS

We have attached a fluorescent probe to a derivative of the famous poison ivy allergen (ref. 7) This is the following tris-bipyrydyl Ruthenium complex.



Model labelling with deuterium was effected by reducing vinylsulfoxides derived from alantolactone and isoanlantolactone, two sesquiterpene α-methylene-γ-butyrolactones with deuterium borohydride (ref. 8). The reaction gave good yields of the deuterated lactones and always proceeded with predominant (2/3) retention of configuration (Scheme I).



Scheme I Deuteration of a vinyl sulfoxide derivative of alantolactone.

SYNTHESIS OF MODEL SKIN HAPTENS

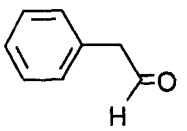
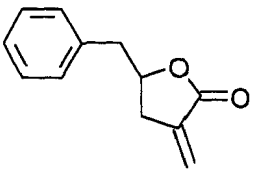
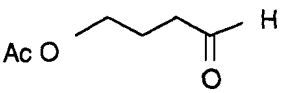
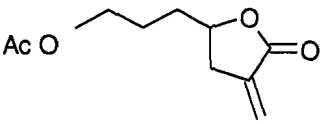
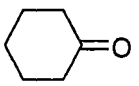
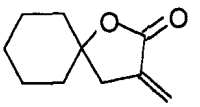
In order to try and understand structural factors associated with the mechanism of allergic contact dermatitis (ACD), we have synthesized a number of allergens and model allergens.

We have studied in particular α-methylene-γ-butyrolactones with different substituents. For many of the lactones, the main sythetic route used was Reformatsky reaction with methyl bromomethymethacrylate (ref.9). However, it is not always convenient to use the usual basic conditions and require in particular for good yields nonenolizable ketones.

We have therefore developed modified Reformatsky conditions in order to be able to use either methacrylic acid or methacrylates. Thus in a method (A) using the

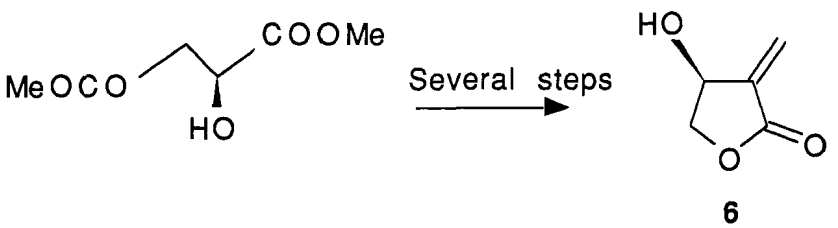
bromomethacrylate ester, a 5:2 THF/Water solvent mixture saturated with ammonium chloride was used. In a second method (B), bromomethacrylic acid could be used directly in a THF-water mixture saturated with ammonium chloride and containing triethylamine. The yields were superior to the ones described in the literature (Table 1).

Table 1. Reformatsky synthesis of α-methylene-γ-butyrolactone (Yields in isolated products)

Substrate	Product	Yields Methods A	B	lit.
		75	52	15
		70	--	12
		77	47	31

Recently, we have used stannous chloride and amberlyst 15 catalysis to prepare a number of α-methylene-γ-butyrolactones under acidic conditions; yields of α-methylene-γ-butyrolactones ranged from 65 to 92 % with aldehydes(ref. 10). None of these methods is suitable for the synthesis of optically active lactones. For this purpose, we used chirons from different sources.

Thus, a number of β-substituted-α-methylene-γ-butyrolactones have been prepared. Both natural tulipalin B **6** and its unnatural enantiomer were synthesized from (-)- S- and (+)-R-methyl lactates respectively (Scheme II) (ref.11).



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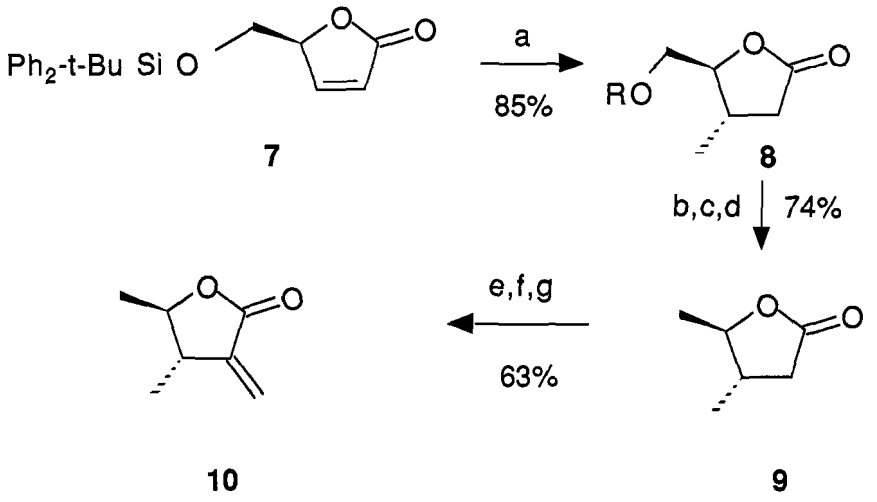
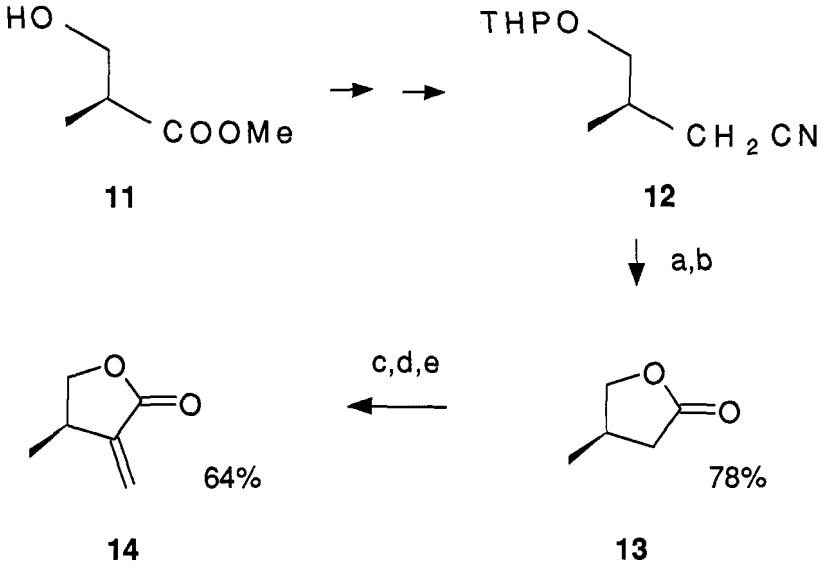
Scheme II Synthesis of (+)-Tulipalin B **6**

(+)-*trans*-β,γ-Dimethyl α-methylene-γ-butyrolactone **10** was readily prepared from γ-t-butyl-diphenylsilyloxymethyl-γ-butenolide **7** obtained in four steps from L-glutamic acid. Michael addition of lithium dimethyl cuprate resulted in high diastereofacial differenciation, resulting in 85% yield of pure enantiomer **8** with the silyl and methyl group *trans* to each other. Classical desilylation, followed by bromination of the γ-hydroxymethyl group and reduction by tributyltin hydride gave β,γ-dimethyl-γ-butyrolactone **9.** The α-methylene group was introduced by LDA treatment followed by reaction with Eschenmoser salt and DBU (Scheme III) (ref.12).



Scheme III Synthesis of *trans*-β, γ-dimethyl-α-methylene-γ-butyrolactone **10** a:a a: Me_2CuLi (R=Ph_2(t-Bu)Si); b:$FNBu_4$; c: CBr_4, PPh3; d:Bu_3SnH, AIBN; e: LDA, H_2CNMe_2I; f: MeI; g: DBU

β–Methyl α-methylene-γ-butyrolactone **14** was synthesized in 5 steps (ref.12) from the known nitrile **12** obtained from methyl β-hydroxybutyrate **11** by Mori's procedure (ref.13) in an overall 82% yield. Hydrolysis of nitrile **12**, followed by deprotection of the alcohol and cyclization with HCl afforded lactone **13** in 78% yield. The α-methylene group was introduced as above with an overall 64% yield (Scheme IV),



a: KOH; b: HCl; c: LDA, $H_2C{=}NMe_2I$; d: MeI; e: $NaHCO_3$

Scheme IV Synthesis of β-methyl–α–methylene-γ-butrolactone **14** from methyl β-hydroxybutyrate

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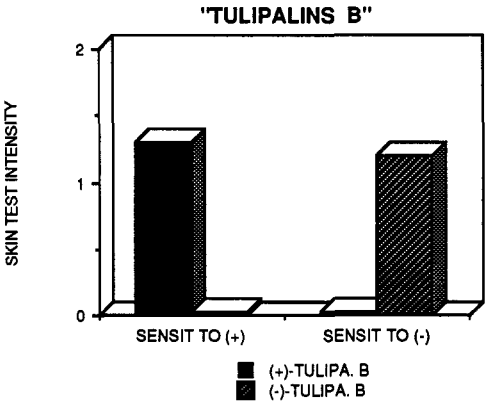
BIOLOGICAL ACTIVITY OF α-METHYLENE-γ-BUTYROLACTONES

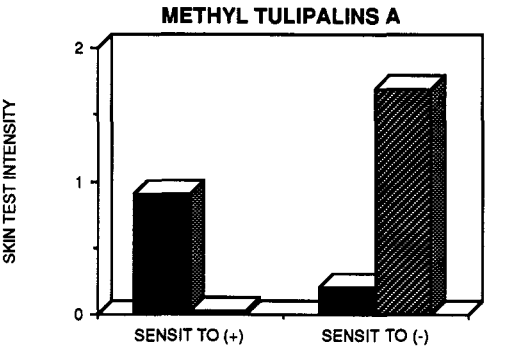
Determination The biological activity of the haptens was determined by *in vivo* methods, using classical sensitization methods in the guinea pig (ref.14) and in mice. In the guinea-pig, the induction of allergy (i.e.sensitization) was effected by injecting intradermally, in the nuchal region of the animal, a 1:1 mixture of Freund complete adjuvant (FCA) and of saline, containing the hapten in varying concentrations. This was repeated three times. Then, the animal, after a 2 weeks rest, was tested epicutaneously by depositing an olive oil:acetone dispersion of the hapten on a measured circular area of the back of the animal. After 24h (but also after 48 and 72 hours), the test was read and graded in a 0-3 scale according to the intensity of the skin reaction (erythema or redness and edema or swelling). The results presented in the different Tables are the averages of the reaction of groups containing 8 to 10 animals and are statistically significant at a $p<0.01$ level.

Results: β-substituted-α-methylene-γ-butyrolactones

These were found to be significant sensitizers (ref. 14) , contrary to reports of the literature (ref.16). In particular, β-hydroxy lactones such as tulipalin B **6**, were as good haptens as the unsubstituted methylenelactones . We sensitized two groups of animals to both natural and unnatural enantiomers of tulipalin B. There was no cross-reaction between the two groups: (+)-tulipalin B-sensitized animals did not react to (-)-tulipalin B and reciprocally (Graph 1). Thus, molecular recognition of these allergens is **enantiospecific**. The same was found for (+)- and (-)-β-methyl-α-methylene-γ-butyrolactones **14** (Graph 2) (ref. 12). This was also true for sesquiterpene lactones from a liverwort, *Frullania:* there is no cross-reactions between the two enantiomers which occur in two different species, namely (+)- and (-)-frullanolides (ref. 17).



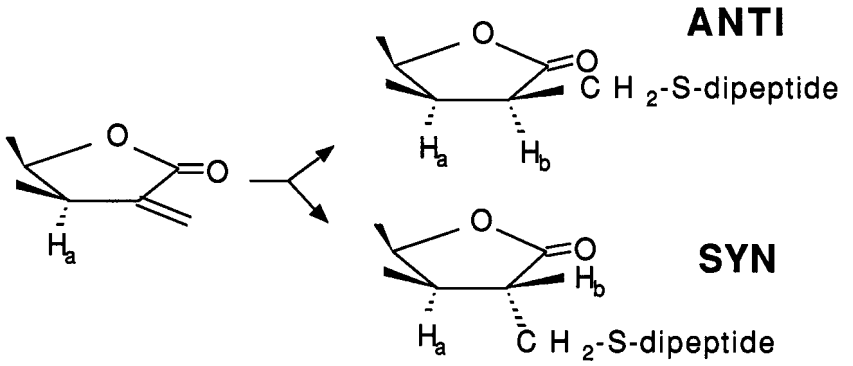
Graph.1 Skin test reactions to (+)- and tulipalins B. No cross-reaction was observed.



Graph 2. Skin test reactions(-)- to (+)- and (-)-β-methyl-α-methylene-γ-butyrolactones.No cross-reaction occurred.

AN *IN VITRO* MODEL OF THE ENANTIOSPECIFICITY OF MOLECULAR RECOGNITION OF β-SUBSTITUTED-α-METHYLENE-γ-BUTYRO-LACTONES

Is it possible to predict the specificity of the skin reaction to β-substituted-α-methylene-γ-butyrolactones? In order to address this question and to try and find a chemical model of protein-aminoacids-hapten reactions, we have prepared a dipeptide, BOC-L-Cys(SH)-Ala-OMe and reacted it with the different enantiomeric lactones according to Scheme V.



Scheme V Reaction of BOC-Cys(SH)-Ala-OMe with α-methylene-γ-butyrolactones.

The reaction conditions were 0.2 M sodium phosphate buffer at pH 7.4 containing absolute methanol to dissolve the dipeptide and the lactone. The results are shown in Table 2.

Thus, when the nucleophilic addition is stereoselective, with high anti/syn ratios, the reaction on the skin is specific and no cross-reaction is observed (i.e. a (+)-enantiomer sensitized animal does not recognize the (-)-enantiomer). When the dipeptide addition is not or poorly stereoselective, the reaction in the skin is not selective: (+)-enantiomers sensitized animals recognize the (-)-enantiomer and conversely. Selectivity of the skin reaction and of the Michael addition of the BOC-Cys(SH)-Ala-OMe peptide to methylenelactones are parallel (ref. 18). Therefore, this model dipeptide although a very rough image of what may happen in skin between nucleophilic proteins and incoming haptens, gives an idea about the selectivity of *in vivo* molecular recognition .

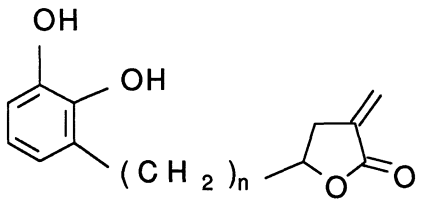
Table 2. Results of addition of BOC-Cys(SH)-Ala-OMe to α-methylene-γ-butyrolactones and of skin tests in guinea-pigs.

γ-LACTONE	Anti-Syn ratio	Average skin reaction to enantiomers[a]	
		(+)	(-)
(+)-β-methyl-	n d	0.9	0
(-)-β-methyl-	9.5/1	0.2	1.7
(+)-γ-methyl	6/1	1.3	0.4
(-)-γ-methyl	2/1	0.8	0.9
(+)-Frullanolide	ANTI	2.0	0.2
(-)-Frullanolide	ANTI	0.3	1.8

[a] Average of skin test intensities varying from 0 to 3 (very strong)

CONCLUSION

The aim of this work was to try and predict adverse skin reactions due to natural and synthetic substances. Some conclusions can be drawn: 1) molecular recognition of haptens by the skin seems enantioselective 2) a chemical model of the supposed *in vivo* protein-hapten interaction gives satisfactory results. Finally, some compounds, not described in this paper seem to have promising properties for inducing specific skin tolerance to some chemicals, such as, for instance, the double-headed hapten below which induces ACD to the pyrocatechol ("poison ivy like") end and tolerance to the α-methylene-γ-butyrolactone ("tulipalin like") end (ref. 19).



Acknowledgement

The efficient and skillful contributions of my coworkers, whose name is mentioned in the references is gratefully acknowledged.

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