ROESY. The determination of the geometry of A / B and ROE and ROESY experiment on proton H-25 and H-5; geometry B / C trial ROE and ROESY on H-26; geometry C / D with irradiated H-27, whereas the D / E with experimental H-28 and H-18. The technique is based on the geometry of A / B; B / C; C / D and D / E can be determined. For example geometry D / E to triterpene Oleana, atom C-28 is above the ring plane (β) so that the C-28 to comparators in the trial ROE and ROESY. If the irradiation is given to H-18 and the result is an effect or no effect on the H-28. If the irradiation of H-18 shows the influence on the H-28, it is a field with HAB-28, which means the geometry of D / E is cis, and if it does not give effect to the opposite situation, namely D / E is trans. Cis position (Figure 4).

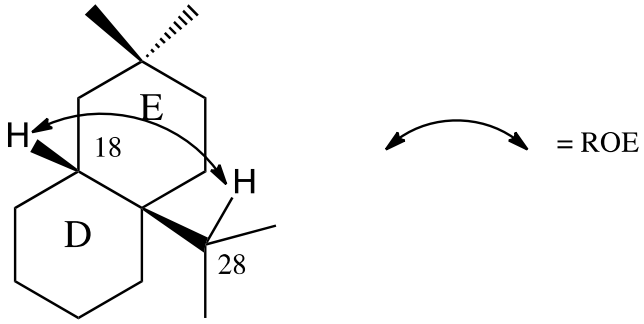

Figure 4. Determination of cis ring geometry D / E saponin triterpene

ROE experiments were also conducted on the configuration of substituents that can be determined appropriately. Conclusion aglycone of the hypothetical can be the figure 5.

d. Determination of acyl substituent

The acyl group is the result of the reaction of the hydroxyl substituent aglycon with carboxylate form acyl as an ester. Their esters have been indicated in the FTIR spectrum that is the uptake 1306 - 1155 cm^{-1} with weak intensity and forms a wide band, but the uptake may also indicate the presence of sugar, and piran ether so that more information is needed. Ascertainment of their substituents acyl saponin is very important because it allows the determination of the amount of monosaccharides a saponin, which is based on the number of C atoms that have been identified through C NMR spectra 1D including DEPT techniques. Total atom C common steroid aglycone is 27 to aglycon colestan, furostan, and spirosolan (saponins alkaloids), 26 for spirostan and solanidine (saponins alkaloids). Furthermore, the number of C atoms for common triterpene saponins is 30. If the number of C atoms by ^{13}C NMR spectra 1D and DEPT> 30, then the excess is a substituent. If a triterpene saponins have 52 carbon atoms based on the analysis of ^{13}C NMR spectrum of which there are atom C with a chemical shift of about 35 ppm indicate the C atoms that there are no substituents (Gaidi et al., 2000; Sanchez et al., 2000; Rahman et al. , 2000), if there is a shift in the atom C with 60-65 ppm indicate the C atoms are hydroxyl substituent (Cadre et al., 2000). If a C aglycone substituted sugar or acid will have a greater shift paramagnetic because more electronegative than hydroxyl mainly sugar up to 185 ppm (Jiang et al., 1999), and, if substituted acids at the C substituents hydroxyl there will be a shift in the chemical atom C of the > 65 ppm and <100 ppm (Qiu et al., 2000). The existence of an acyl substituent at C atom aglycone followed by HMBC techniques that correlate the distance between the aglycone signal H atom by atom C also acyl or C atom by atom H aglycone acyl substituent. If an acyl group as a substituent on a chemical shift, in general, have higher because more electronegative or a C carbonyl which is about 170-180 ppm (Qiu et al., 2000). Signal carbonyl C atom acyl proton signal correlated with a number of acyls ranged from 1.00 to 1.5 ppm (Qiu et al., 2000). Thus the structure of the acyl groups substituted on the C atom saponin aglycone can be determined either with 1D NMR spectra; HMBC NMR techniques; TOCSY, HH-COSY, and HMQC / HSQC.

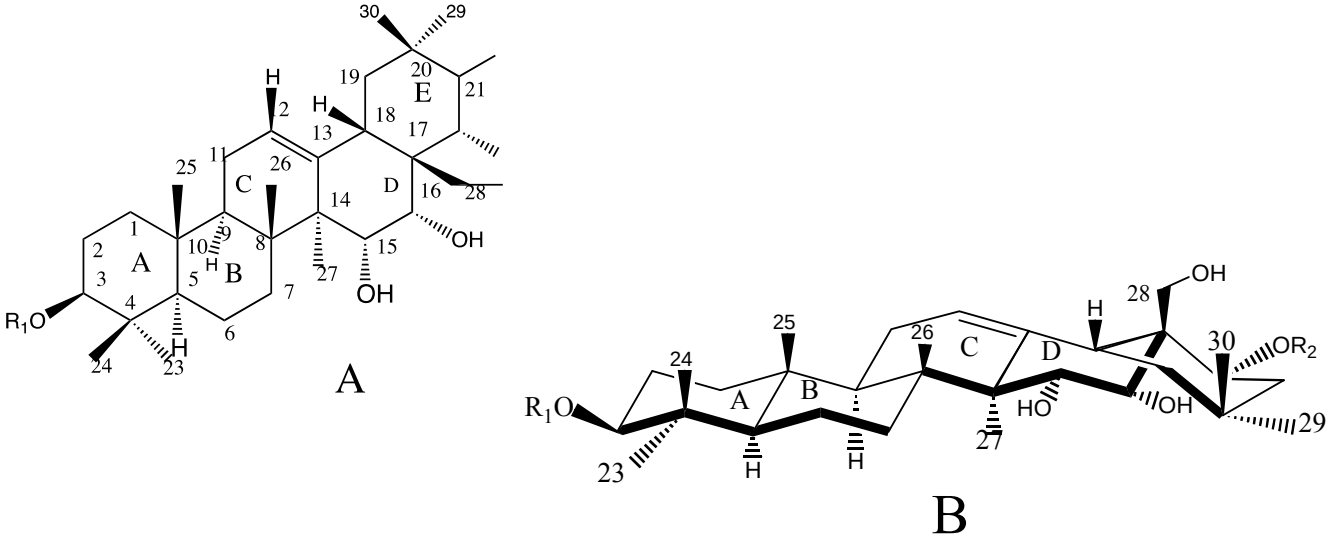

Figure 5. Determination technique of the geometry and conformation ring as well as the configuration of substituents on triterpene sapogenin Oleana

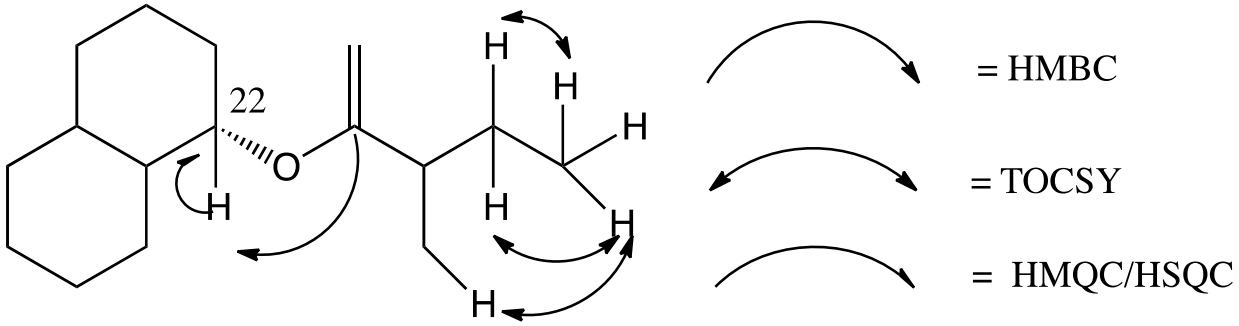

Figure 6. Acyl substituent structure determination technique of in saponins

e. Determination of Monosaccharides

Monosaccharides commonly found as part of saponin sugar is D-glucose, D-galactose, D-glucuronic acid, D-ribose, D-xylose, L-arabinose, L-fucose, and L-rhamnose. The parts of the sugar atom substituted at C-3 or more C atoms with α or β-position anomeric. Parts sugar or monosaccharide that can be bound to different C atoms in one type of saponin. If the sugar part is only attached to the C atom is called monodesmosidic, on two different C atoms called the bidesmosidic, and three different C atoms called the tridesmosidic. Until now saponins found still limited to tridesmosidic and undiscovered tetradesmosidic.

Type of saponin monosaccharides are hexoses with 6 C atoms except D-glucuronic acid 7 atom C. In theory, if a triterpene saponin is bound to one molecule monosaccharide will find 36-37 signal atom C; if two sugar molecules are 42-43 signal atom C; and if three molecules will be found 48-49 atom signal C. That situation may occur if there is no signal of the C atoms are overlapping, so that the number of atoms C signal is not always used as a primary consideration. Determining the type starts with the determination of monosaccharides saponin aglycone a C atom bound monosaccharide and the next monosaccharide determination of C atoms in the atom C monosaccharide directly attached to the aglycone, and so on. Thus the determination of the monosaccharide and aglycone C atom bound monosaccharide place gradually. Monosaccharide bound to the C atom aglycone symbolize with M-1; monosaccharide bound to the M-1 symbolize with M-2 and so on. If saponin is bidesmosidic or tridesmosidic it can symbolize with M-2 'and M-3 "; whereas monosaccharide bound to the M-2 symbolize with M-2'-1; bound on M-2'-1 symbolizes with M-2'-1 'and so on. The symbol can be made freely by the researchers in accordance with the level of convenience that were understood. Determination of atom C-section where the bound sugar next part sugar is a monosaccharide structure determination so well known as type and structure of the monosaccharide sugars as part of saponin.

1) Determination of Monosaccharide M-1

Monosaccharides M-1 is the monosaccharide directly attached to the aglycone of saponins. Determination of the monosaccharide substituents using techniques HMBC by correlating signal aglycone a C atom bound with M-1 H atom monosaccharides, and the C atom at M-1 with the H atom attached to the C atom aglycone. The HMBC information has confirmed the existence of M-1 bound to the C atom aglycone. Configuration or position of the M-1 as a β-glycosidic or α-glycosidic can be known through constant coupling proton atom C aglycone and the C-1 'in M-1, but must be confirmed by techniques ROE and ROESY for H atoms which are unidirectional or not unidirectional the sugar molecules, whereas TOCSY help correlate the influence of H atoms bonded to atom C aglycone with H atoms bonded to atom C sugar or between the H atoms bonded to atoms of C part sugar is by irradiation H atoms. Thus the NMR technique is important for determining the structure of monosaccharides M-1 is HMBC, HMQC / HSQC, HH-COSY, ROE and ROESY, TOCSY. Signal atom C sugar section generally ranges in chemical shift 65-110 ppm; while the proton signal ranges from 3.3 to 5.0 ppm (Hostettmann and Marston, 1995). If there is a signal> 170 ppm should be assumed as a carboxyl C atoms, such as glucuronic acid. The presence of carboxyl groups can also be identified on FTIR absorption as C = O and C-O-C and C-OH carboxyl.

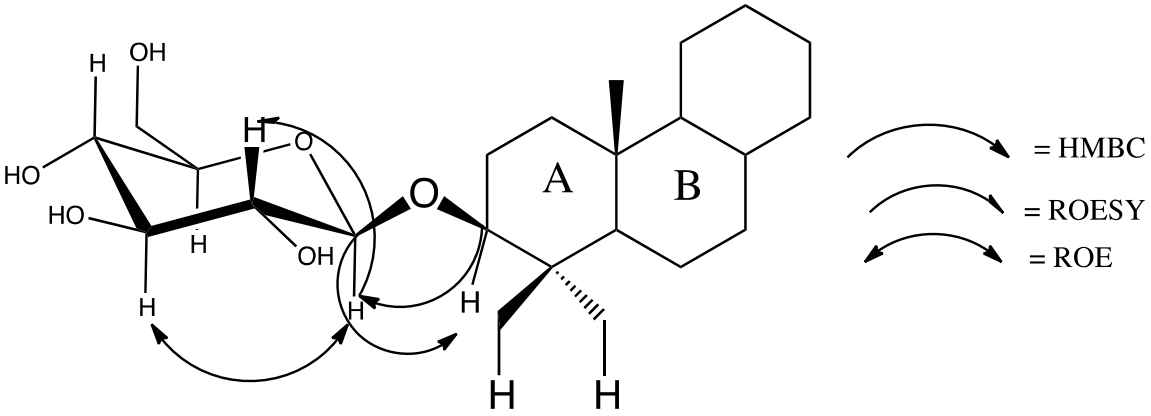

Figure 7. determination technique of the structure of monosaccharides M-1 saponin

2) Determination of Monosaccharide M-2

Monosaccharides M-2 is a monosaccharide molecule bound to the monosaccharide M-1. Possible ties between M-1 and M-2 can be either β or α position to form 1'□2'' or 1'□ 3''. NMR technique of are mainly used to determine the structure and configuration of the M-2 is HMBC, ROE, ROESY, TOCSY enhanced with HH-COSY, HMQC / HSQC. Signal carbon commonly found also range in a chemical shift at 65-110 ppm; while the proton signal ranges from 3.3 to 5.0 ppm (Hostettmann and Marston, 1995). Determining the structure of M-2 is equal to monosalaroda M-3, and so if part sugar saponin consists of 3 or 4 molecules of monosaccharides.

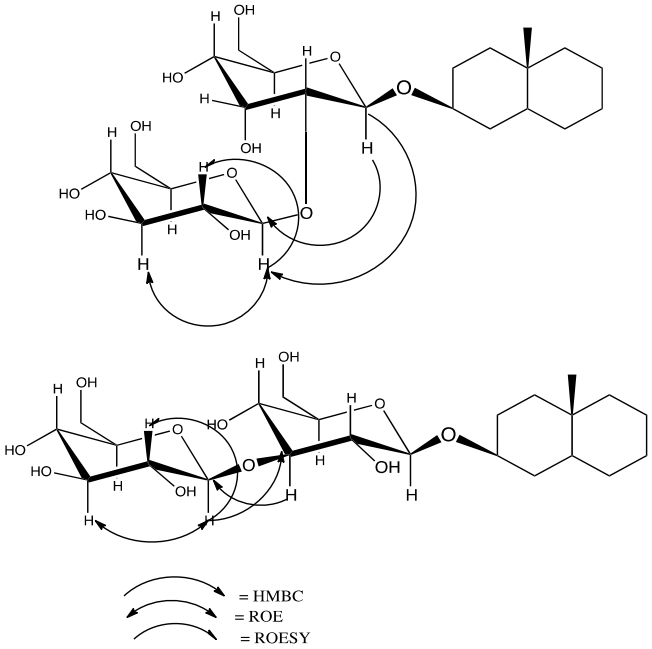

Figure 8. Determination technique for monosaccharides M-2

CONCLUSION

1. Fast technique saponin extraction using methanol-water with a ratio of 7: 3 or 1: 1 and perform the purification of saponin mixture (without other metabolites) using ether were added in a solution containing saponin fractions.
2. Mechanical precise and rapid isolation of saponins are using preparative HPLC column or semi-preparative HPLC eluent with determining the analytical column
3. The appropriate eluent for the separation of saponins by reverse phase technique using preparative HPLC column or semipreparative are methanol-water or methanol-water-acetic acid to very polar saponins
4. Engineering quick determination of the structure of saponin in a way to structure elucidation stages, namely the determination of the aglycone, the determination of the sugar portion consisting of monosaccharides attached to the aglycone, monosaccharides bound monosaccharides attached to the aglycone and so on, as well as the determination of substituents commonly found in saponins such as methyl , metin, acyl, and hydroxyl
5. The main instrument for structure determination is a saponin-2D NMR frequency ≥ 400 MHz with techniques HMBC, HMQC / HSQC, TOCSY, ROE, ROESY, and software such as MS instruments FABMS (ESIMS).
6. NMR techniques in addition to those techniques are also considered very helpful is the DEPT spectrum and NMR-1D
7. FTIR instrument helped to accelerate the general determination that the identified compound is a steroidal glycoside or triterpene glycosides

RECCOMENDATION

Isolation and structure determination of saponin from a natural material of biological directly using extraction techniques that, using preparative HPLC or semipreparative and analytical HPLC for the determination of eluent, while the determination of the structure directly using NMR-2D with a frequency ≥ 400 MHz with techniques HMBC, HMQC / HSQC, ROE , ROESY, TOCSY, and HH-COSY. Such techniques may be assisted by 1D NMR spectra and DEPT techniques, while another important instrument is the MS with FABMS (ESIMS) and FTIR.

ACKNOWLEDGMENT

This article aims to present a technique of isolation and structure elucidation saponins precise, fast and effective, due to the isolation and structure elucidation of saponins known to be very difficult despite the use of separation equipment and high-tech instruments. Therefore I as a reviewer of this article would like to thank all the writers of books reference and author of the scientific article that became the source of this scientific article. The authors are those listed in the bibliography of scientific articles and are also written in the methods section of this article. Hopefully this article useful to researchers saponin especially the students and the services of the authors of articles and books as a source of this article.

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